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Reference Librarian
Biotechnology & Chemical Library
CM1 1E07 – 703-308-4498
jan.delaval@uspto.gov

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=> fil reg
FILE 'REGISTRY' ENTERED AT 15:15:32 ON 08 NOV 2002
USE IS SUBJECT TO THE TERMS OF YOUR STN CUSTOMER AGREEMENT.
PLEASE SEE "HELP USAGETERMS" FOR DETAILS.
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Property values tagged with IC are from the ZIC/VINITI data file
provided by InfoChem.

STRUCTURE FILE UPDATES:    7 NOV 2002  HIGHEST RN 471842-29-2
DICTIONARY FILE UPDATES:   7 NOV 2002  HIGHEST RN 471842-29-2

TSCA INFORMATION NOW CURRENT THROUGH MAY 20, 2002

  Please note that search-term pricing does apply when
  conducting SmartSELECT searches.

Crossover limits have been increased.  See HELP CROSSOVER for details.

Experimental and calculated property data are now available.  See HELP
PROPERTIES for more information.  See STNote 27, Searching Properties
in the CAS Registry File, for complete details:
http://www.cas.org/ONLINE/STN/STNOTES/stnotes27.pdf

=> d ide can l1

L1   ANSWER 1 OF 1  REGISTRY  COPYRIGHT 2002 ACS
RN   11096-26-7  REGISTRY
CN   Erythropoietin (9CI)  (CA INDEX NAME)
OTHER NAMES:
CN   EPO
CN   Epoetin
CN   Epogis S
MF   Unspecified
CI   COM, MAN
LC   STN Files:   ADISNEWS, AGRICOLA, ANABSTR, BIOBUSINESS, BIOSIS, BIOTECHNO,
       CA, CABA, CANCERLIT, CAPLUS, CBNB, CEN, CHEMCATS, CHEMLIST, CIN, CSCHEM,
       DDFU, DIOGENES, DRUGPAT, DRUGU, DRUGUPDATES, EMBASE, IFICDB, IFIPAT,
       IFIUDB, IPA, MEDLINE, MRCK*, NIOSHTIC, PHAR, PROMT, RTECS*, TOXCENTER,
       USPAT2, USPATFULL, VETU
         (*File contains numerically searchable property data)
     Other Sources:   EINECS**
         (**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***
            7102 REFERENCES IN FILE CA (1962 TO DATE)
             161 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
            7105 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE   1:  137:299972

REFERENCE   2:  137:299914

REFERENCE   3:  137:293567

REFERENCE   4:  137:293537

REFERENCE   5:  137:293511

REFERENCE   6:  137:292887

REFERENCE   7:  137:292523
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REFERENCE 8: 137:292492

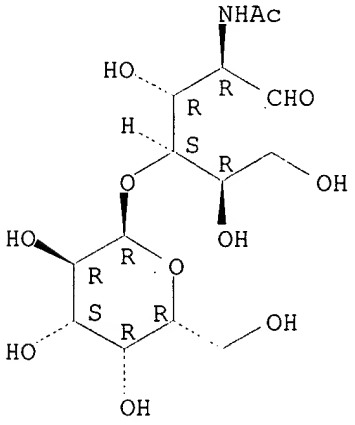
REFERENCE 9: 137:289926

REFERENCE 10: 137:289913

=> d l48 ide can tot

L48 ANSWER 1 OF 5 REGISTRY COPYRIGHT 2002 ACS
RN 205380-69-4 REGISTRY
CN **D-Glucose, 2-(acetylamino)-2-deoxy-4-O-.alpha.-D-galactopyranosyl-(9CI)** (CA INDEX NAME)
FS STEREOSEARCH
MF **C14 H25 N O11**
SR CA
LC STN Files: CA, CAPLUS, USPATFULL

Absolute stereochemistry.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

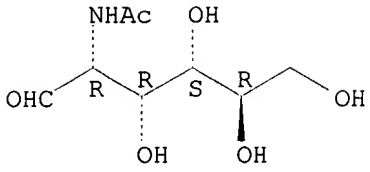
REFERENCE 1: 128:256472

L48 ANSWER 2 OF 5 REGISTRY COPYRIGHT 2002 ACS
RN **112692-64-5** REGISTRY
CN D-Glucose, 2-(acetylamino)-2-deoxy-O-.beta.-D-galactopyranosyl- (9CI) (CA INDEX NAME)
FS STEREOSEARCH
MF C14 H25 N O11
CI IDS
SR CA
LC STN Files: CA, CAPLUS, USPATFULL

CM 1

CRN 7512-17-6
CMF C8 H15 N O6

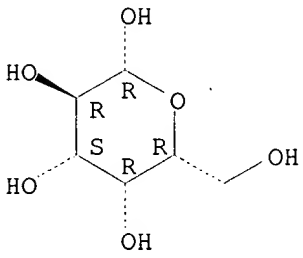
Absolute stereochemistry.



CM 2

CRN 7296-64-2
CMF C6 H12 O6

Absolute stereochemistry.



2 REFERENCES IN FILE CA (1962 TO DATE)
2 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 108:166119

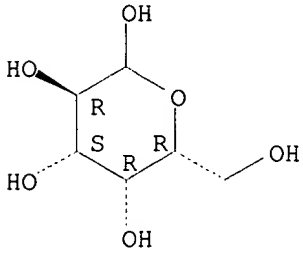
REFERENCE 2: 108:62432

L48 ANSWER 3 OF 5 REGISTRY COPYRIGHT 2002 ACS
RN **99569-97-8** REGISTRY
CN D-Glucose, 2-(acetylamino)-2-deoxy-O-D-galactopyranosyl- (9CI) (CA INDEX NAME)
FS STEREOSEARCH
MF C14 H25 N O11
CI IDS
SR CA
LC STN Files: CA, CAPLUS, USPATFULL

CM 1

CRN 10257-28-0
CMF C6 H12 O6

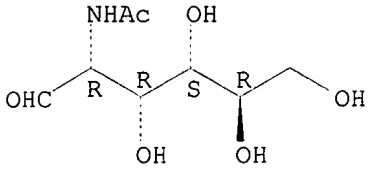
Absolute stereochemistry.



CM 2

CRN 7512-17-6
CMF C8 H15 N O6

Absolute stereochemistry.



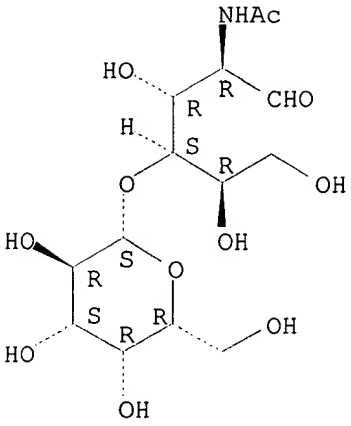
2 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
2 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 113:94401

REFERENCE 2: 104:18088

L48 ANSWER 4 OF 5 REGISTRY COPYRIGHT 2002 ACS
RN 32181-59-2 REGISTRY
CN **D-Glucose, 2-(acetylamino)-2-deoxy-4-O-.beta.-D-galactopyranosyl- (9CI)** (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN **D-Glucosamine, N-acetyl-4-O-.beta.-D-galactopyranosyl- (6CI)**
CN **D-Glucose, 2-acetamido-2-deoxy-4-O-.beta.-D-galactopyranosyl- (7CI, 8CI)**
OTHER NAMES:
CN **2-Acetamido-2-deoxy-4-O-.beta.-D-galactopyranosyl-D-glucose**
CN Lactosamine, N-acetyl-
CN **N-Acetyl-4-O-.beta.-D-galactopyranosyl-D-glucosamine**
CN N-Acetyllactosamine
CN **O-.beta.-D-Galactopyranosyl-(1.fwdarw.4)-2-deoxy-2-acetamido-D-glucose**
AR 4307-58-8
FS STEREOSEARCH
DR 133432-89-0, 98529-93-2
MF **C14 H25 N O11**
CI COM
LC STN Files: AGRICOLA, BEILSTEIN*, BIOBUSINESS, BIOSIS, CA, CANCERLIT, CAOLD, CAPLUS, CASREACT, CHEMCATS, CSCHEM, MEDLINE, MSDS-OHS, PROMT, TOXCENTER, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

668 REFERENCES IN FILE CA (1962 TO DATE)
74 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
671 REFERENCES IN FILE CAPLUS (1962 TO DATE)
38 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

REFERENCE 1: 137:274990

REFERENCE 2: 137:228351

REFERENCE 3: 137:215941

REFERENCE 4: 137:213993

REFERENCE 5: 137:190747

REFERENCE 6: 137:185766

REFERENCE 7: 137:165159

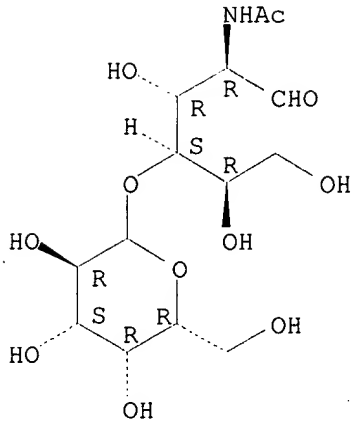
REFERENCE 8: 137:155129

REFERENCE 9: 137:114486

REFERENCE 10: 137:108392

L48 ANSWER 5 OF 5 REGISTRY COPYRIGHT 2002 ACS
RN 5639-05-4 REGISTRY
CN **D-Glucose, 2-(acetylamino)-2-deoxy-4-O-D-galactopyranosyl- (9CI)**
(CA INDEX NAME)
OTHER CA INDEX NAMES:
CN **D-Glucosamine, N-acetyl-4-O-D-galactopyranosyl- (6CI)**
FS STEREOSEARCH
MF **C14 H25 N O11**
LC STN Files: BEILSTEIN*, CA, CAOLD, CAPLUS
(*File contains numerically searchable property data)

Absolute stereochemistry.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

2 REFERENCES IN FILE CA (1962 TO DATE)
2 REFERENCES IN FILE CAPLUS (1962 TO DATE)
1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

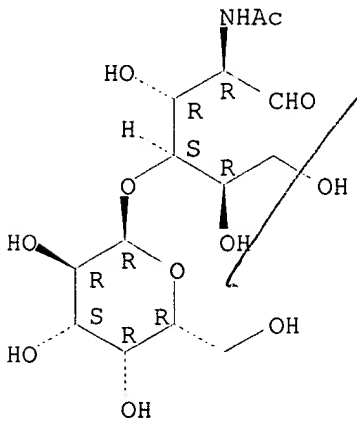
REFERENCE 1: 118:213410

REFERENCE 2: 117:207503

=> d ide can tot

L92 ANSWER 1 OF 3 REGISTRY COPYRIGHT 2002 ACS
RN 205380-69-4 REGISTRY
CN **D-Glucose, 2-(acetylamino)-2-deoxy-4-O-.alpha.-D-galactopyranosyl-**
(9CI) (CA INDEX NAME)
FS STEREOSEARCH
MF **C14 H25 N O11**
SR CA
LC STN Files: CA, CAPLUS, USPATFULL

Absolute stereochemistry.



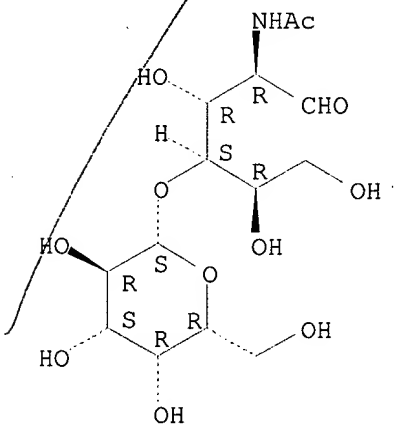
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 128:256472

L92 ANSWER 2 OF 3 REGISTRY COPYRIGHT 2002 ACS
RN 32181-59-2 REGISTRY
CN **D-Glucose, 2-(acetylamino)-2-deoxy-4-O-.beta.-D-galactopyranosyl- (9CI)** (CA INDEX NAME)
OTHER CA INDEX NAMES:
CN **D-Glucosamine, N-acetyl-4-O-.beta.-D-galactopyranosyl- (6CI)**
CN **D-Glucose, 2-acetamido-2-deoxy-4-O-.beta.-D-galactopyranosyl- (7CI, 8CI)**
OTHER NAMES:
CN **2-Acetamido-2-deoxy-4-O-.beta.-D-galactopyranosyl-D-glucose**
CN Lactosamine, N-acetyl-
CN **N-Acetyl-4-O-.beta.-D-galactopyranosyl-D-glucosamine**
CN N-Acetyllactosamine
CN **O-.beta.-D-Galactopyranosyl-(1.fwdarw.4)-2-deoxy-2-acetamido-D-glucose**
AR 4307-58-8
FS STEREOSEARCH
DR 133432-89-0, 98529-93-2
MF **C14 H25 N O11**
CI COM
LC STN Files: AGRICOLA, BEILSTEIN*, BIOBUSINESS, BIOSIS, CA, CANCERLIT, CAOLD, CAPLUS, CASREACT, CHEMCATS, CSCHEM, MEDLINE, MSDS-OHS, PROMT, TOXCENTER, USPATFULL
(*File contains numerically searchable property data)

Absolute stereochemistry.



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

668 REFERENCES IN FILE CA (1962 TO DATE)
74 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
671 REFERENCES IN FILE CAPLUS (1962 TO DATE)
38 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

REFERENCE 1: 137:274990

REFERENCE 2: 137:228351

REFERENCE 3: 137:215941

REFERENCE 4: 137:213993

REFERENCE 5: 137:190747

REFERENCE 6: 137:185766

REFERENCE 7: 137:165159

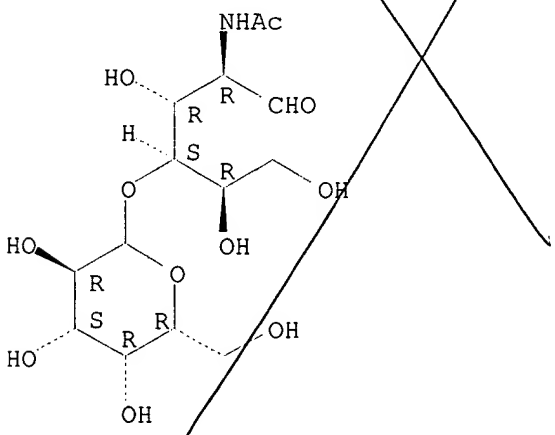
REFERENCE 8: 137:155129

REFERENCE 9: 137:114486

REFERENCE 10: 137:108392

L92 ANSWER 3 OF 3 REGISTRY COPYRIGHT 2002 ACS
RN 5639-05-4 REGISTRY
CN **D-Glucose, 2-(acetylamino)-2-deoxy-4-O-D-galactopyranosyl- (9CI)**
(CA INDEX NAME)
OTHER CA INDEX NAMES:
CN **D-Glucosamine, N-acetyl-4-O-D-galactopyranosyl- (6CI)**
FS STEREOSEARCH
MF **C14 H25 N O11**
LC STN Files: BEILSTEIN*, CA, CAOLD, CAPLUS
(*File contains numerically searchable property data)

Absolute stereochemistry.



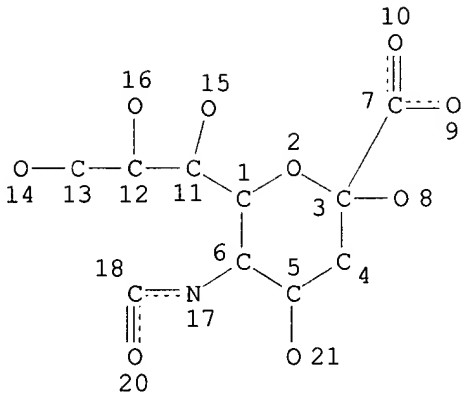
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

2 REFERENCES IN FILE CA (1962 TO DATE)
2 REFERENCES IN FILE CAPLUS (1962 TO DATE)
1 REFERENCES IN FILE CAOLD (PRIOR TO 1967)

REFERENCE 1: 118:213410

REFERENCE 2: 117:207503

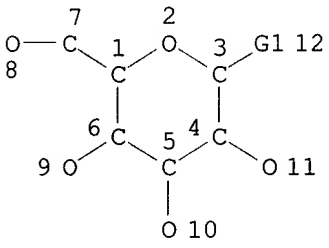
=> d sta que l85
L49 5 SEA FILE=REGISTRY ABB=ON PLU=ON (112692-64-5/CRN OR 205380-69-4/CRN OR 32181-59-2/CRN OR 5639-05-4/CRN OR 99569-97-8/CRN)
L63 STR

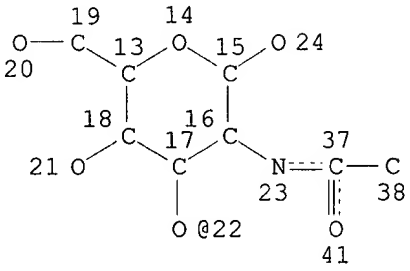


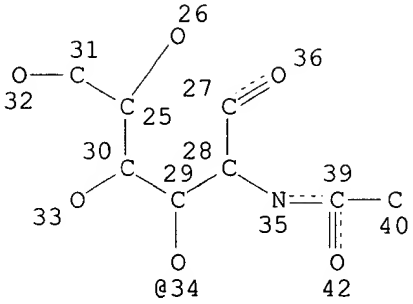
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 20

STEREO ATTRIBUTES: NONE
L64 9510 SEA FILE=REGISTRY SSS FUL L63
L65 121 SEA FILE=REGISTRY ABB=ON PLU=ON L64 AND PMS/CI
L66 9387 SEA FILE=REGISTRY ABB=ON PLU=ON L64 NOT (L49 OR L65)
L67 STR







VAR G1=22/34
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 42

STEREO ATTRIBUTES: NONE

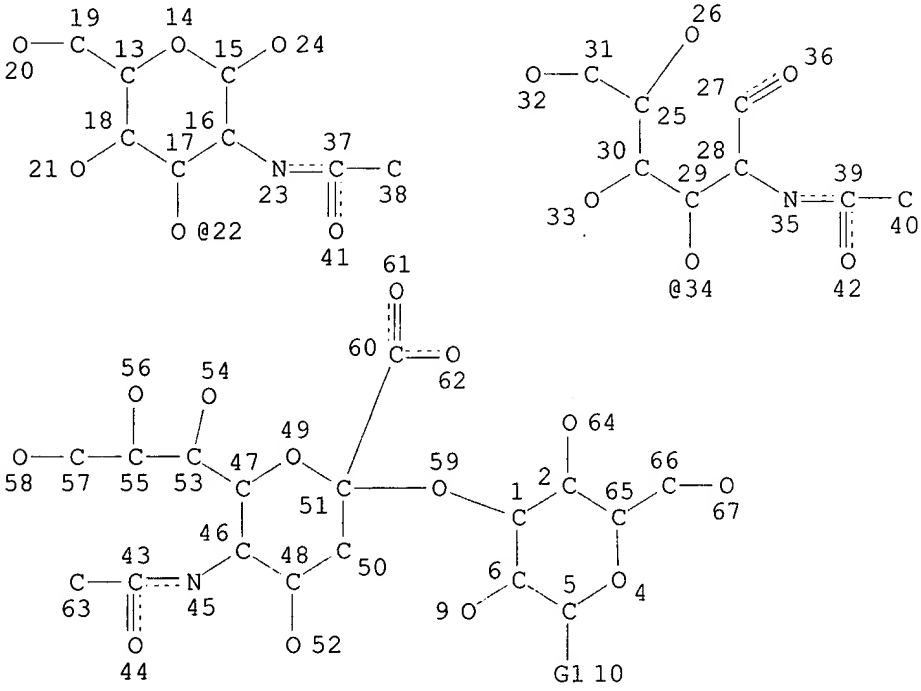
L69 1402 SEA FILE=REGISTRY SUB=L66 SSS FUL L67
L70 STR

Cb 1

NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 1

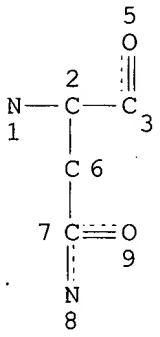
STEREO ATTRIBUTES: NONE
L72 373 SEA FILE=REGISTRY SUB=L69 SSS FUL L70
L73 1029 SEA FILE=REGISTRY ABB=ON PLU=ON L69 NOT L72
L81 STR



VAR G1=22/34
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 62

STEREO ATTRIBUTES: NONE
L82 511 SEA FILE=REGISTRY SUB=L73 SSS FUL L81
L83 STR



NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 8

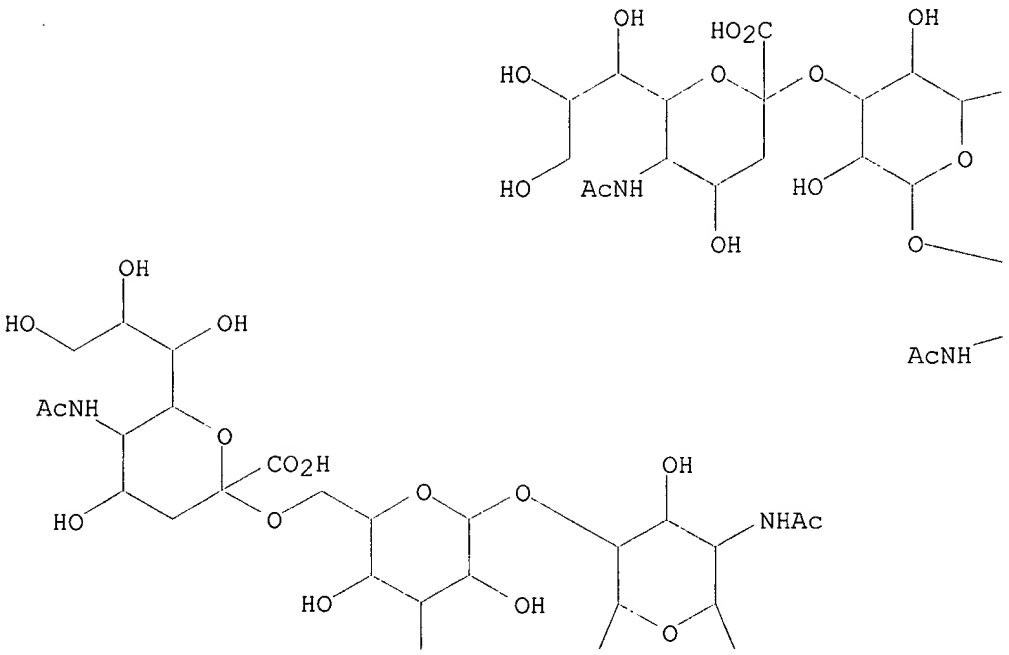
STEREO ATTRIBUTES: NONE
L85 1 SEA FILE=REGISTRY SUB=L82 SSS FUL L83

100.0% PROCESSED 1 ITERATIONS 1 ANSWERS
SEARCH TIME: 00.00.01

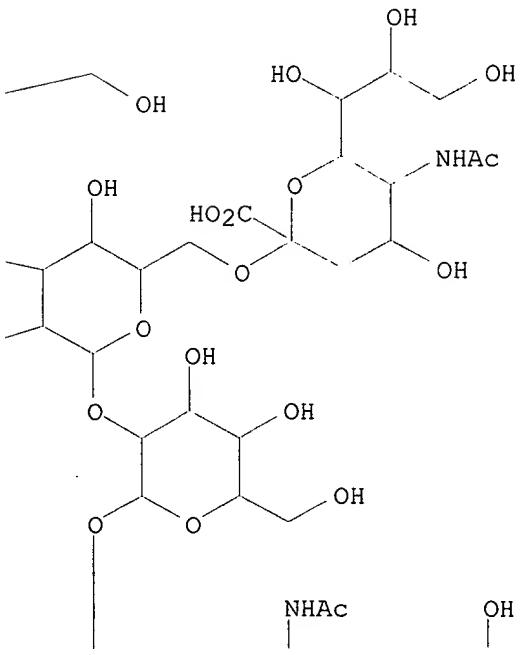
=> d ide can l85

L85 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2002 ACS
RN 97534-26-4 REGISTRY
CN L-Asparagine, N-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-.beta.-D-galactopyranosyl-(1.fwdarw.3)]-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.6)-O-.beta.-D-galactopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl]- (9CI) (CA INDEX NAME)
MF C99 H161 N9 O72
SR CA
LC STN Files: CA, CAPLUS

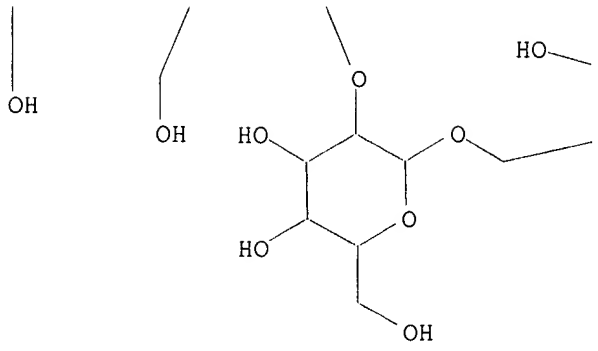
PAGE 1-A



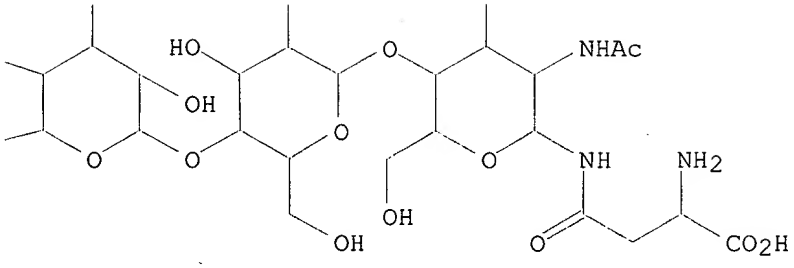
PAGE 1-B



PAGE 2-A



PAGE 2-B



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

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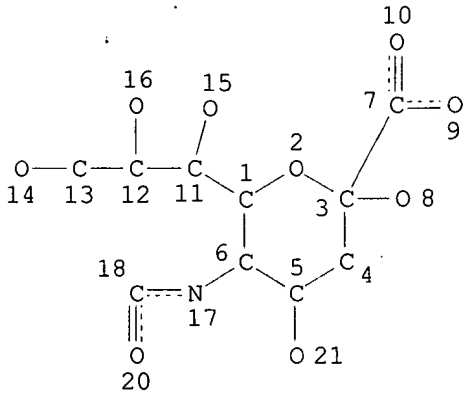
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 103:67027

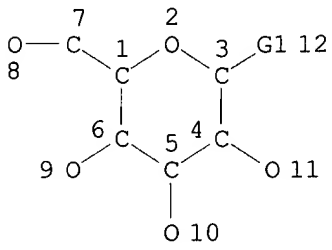
=> d sta que l89
L49 5 SEA FILE=REGISTRY ABB=ON PLU=ON (112692-64-5/CRN OR 205380-69-4/CRN OR 32181-59-2/CRN OR 5639-05-4/CRN OR 99569-97-8/CRN)
L63 STR

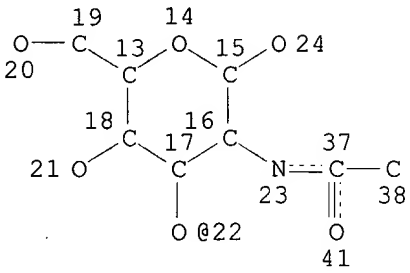


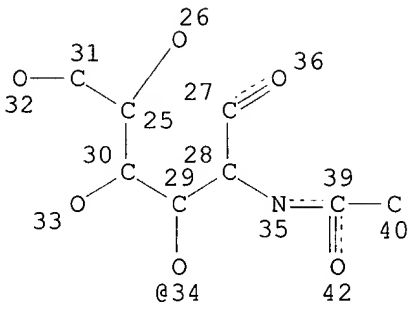
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 20

STEREO ATTRIBUTES: NONE
L64 9510 SEA FILE=REGISTRY SSS FUL L63
L65 121 SEA FILE=REGISTRY ABB=ON PLU=ON L64 AND PMS/CI
L66 9387 SEA FILE=REGISTRY ABB=ON PLU=ON L64 NOT (L49 OR L65)
L67 STR







VAR G1=22/34
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 42

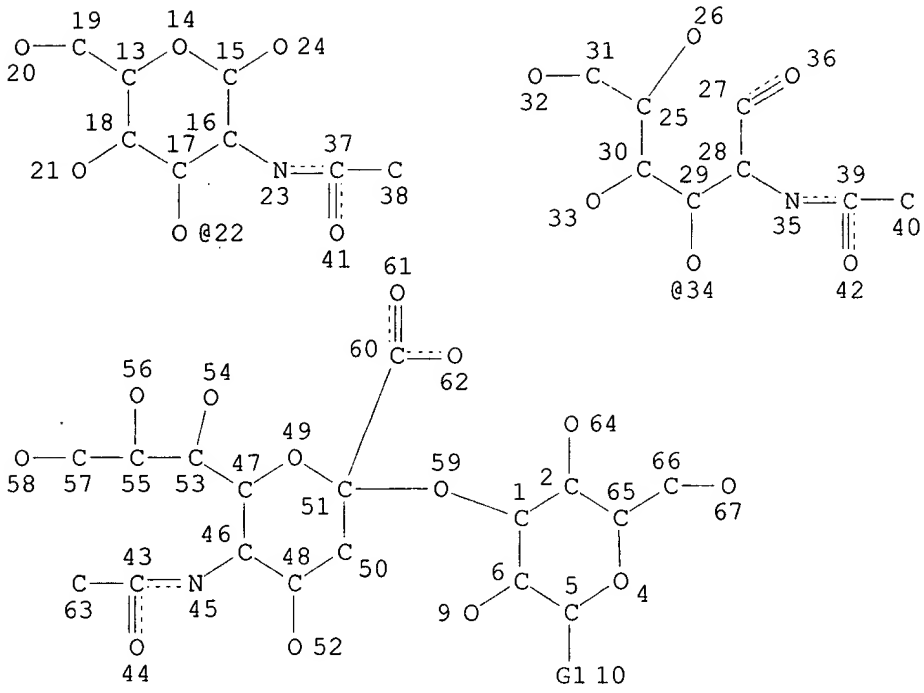
STEREO ATTRIBUTES: NONE
L69 1402 SEA FILE=REGISTRY SUB=L66 SSS FUL L67
L70 STR

Cb 1

NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 1

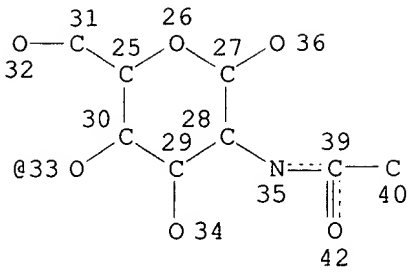
STEREO ATTRIBUTES: NONE
L72 373 SEA FILE=REGISTRY SUB=L69 SSS FUL L70
L73 1029 SEA FILE=REGISTRY ABB=ON PLU=ON L69 NOT L72
L81 STR

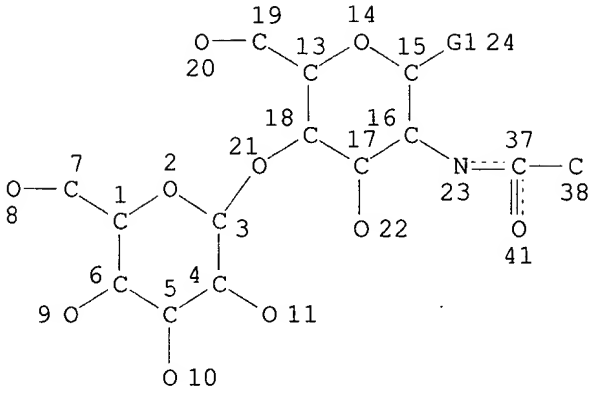


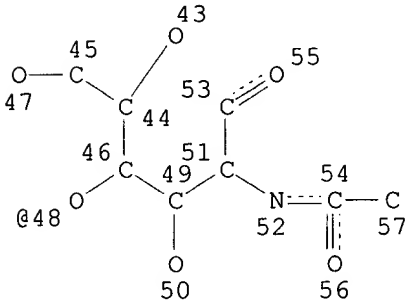
VAR G1=22/34
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 62

STEREO ATTRIBUTES: NONE
L82 511 SEA FILE=REGISTRY SUB=L73 SSS FUL L81
L86 STR



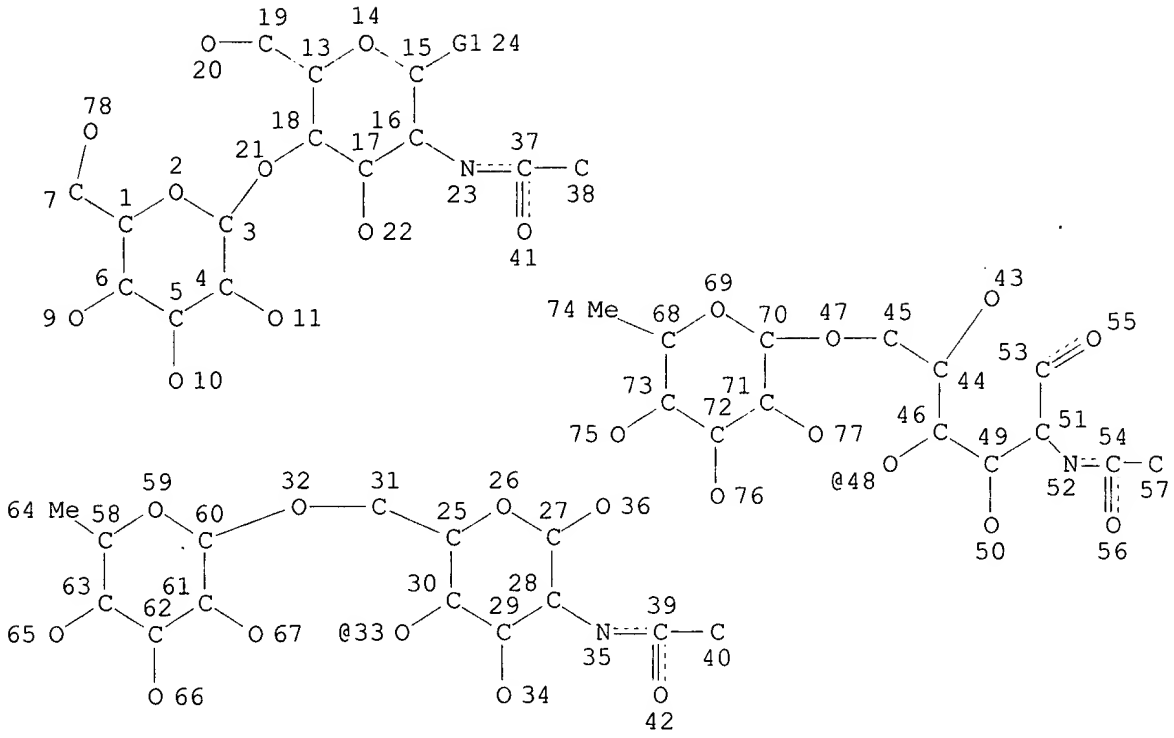




VAR G1=33/48
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 56

STEREO ATTRIBUTES: NONE
L87 48 SEA FILE=REGISTRY SUB=L82 SSS FUL L86
L88 STR



VAR G1=33/48
NODE ATTRIBUTES:
DEFAULT MLEVEL IS ATOM
DEFAULT ECLEVEL IS LIMITED

GRAPH ATTRIBUTES:
RING(S) ARE ISOLATED OR EMBEDDED
NUMBER OF NODES IS 76

STEREO ATTRIBUTES: NONE
L89 18 SEA FILE=REGISTRY SUB=L87 SSS FUL L88

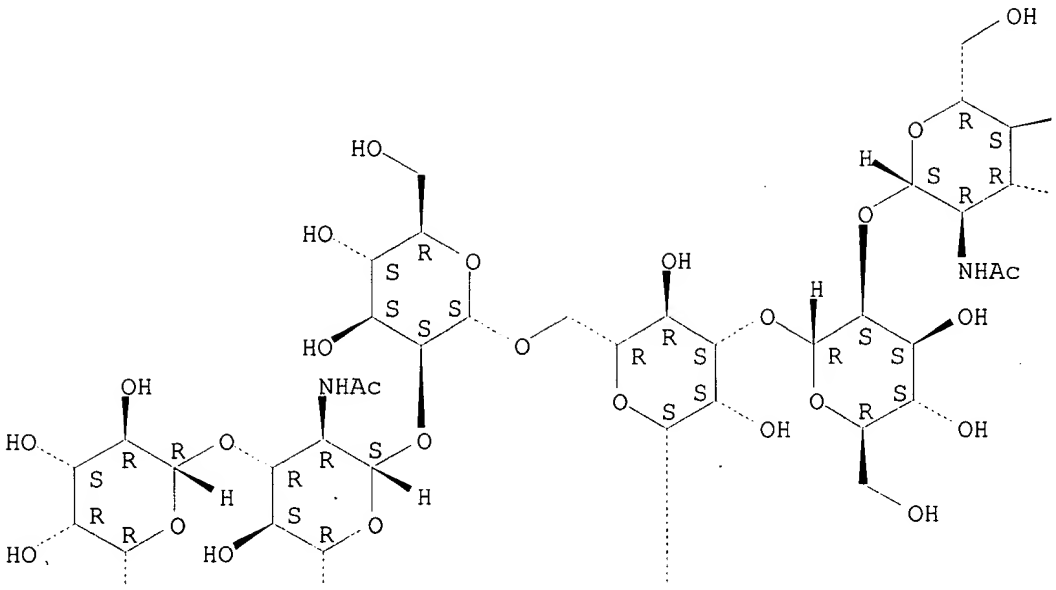
100.0% PROCESSED 48 ITERATIONS 18 ANSWERS
SEARCH TIME: 00.00.03

=> d ide can l89 tot

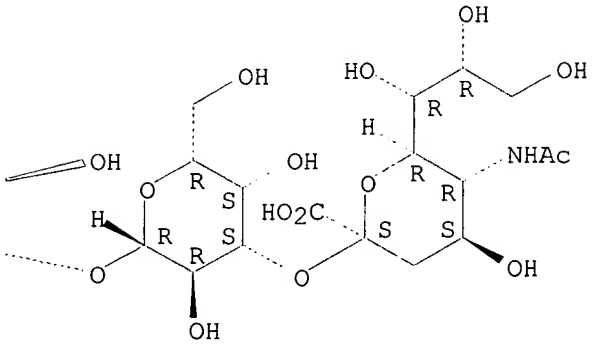
L89 ANSWER 1 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 173557-99-8 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
FS STEREOSEARCH
MF C79 H131 N5 O58
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

Absolute stereochemistry.

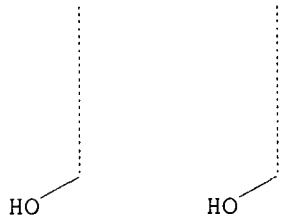
PAGE 1-A

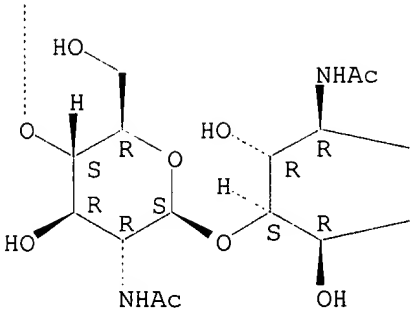


PAGE 1-B

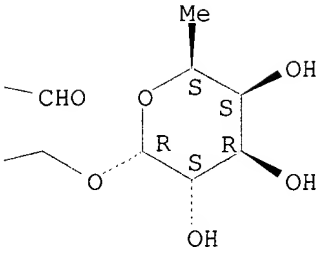


PAGE 2-A





PAGE 2-B



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

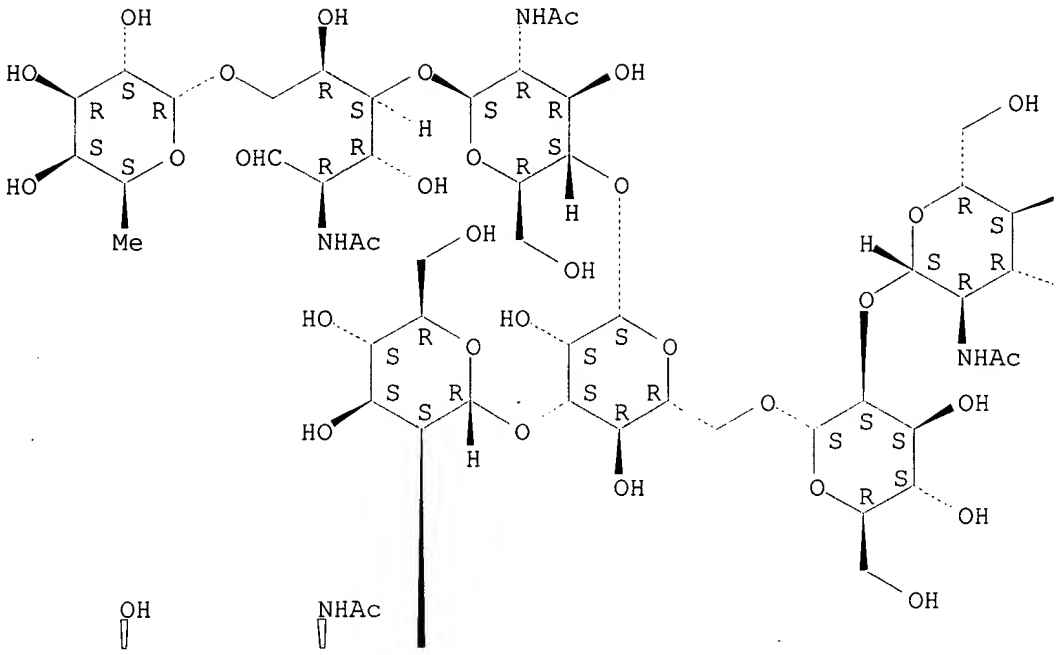
1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 124:169248

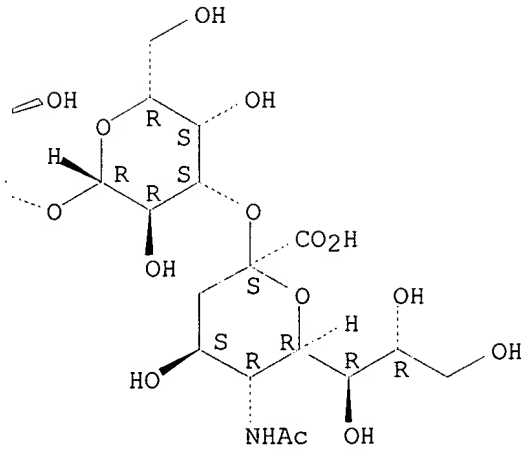
L89 ANSWER 2 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 173557-98-7 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.6)-O-[O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.3)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
FS STEREOSEARCH
MF C79 H131 N5 O58
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

Absolute stereochemistry.

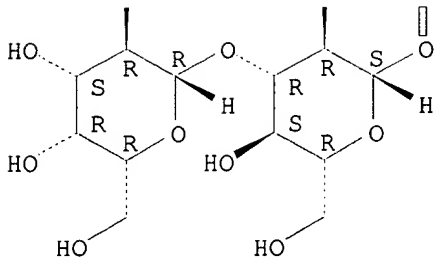
PAGE 1-A



PAGE 1-B



PAGE 2-A



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

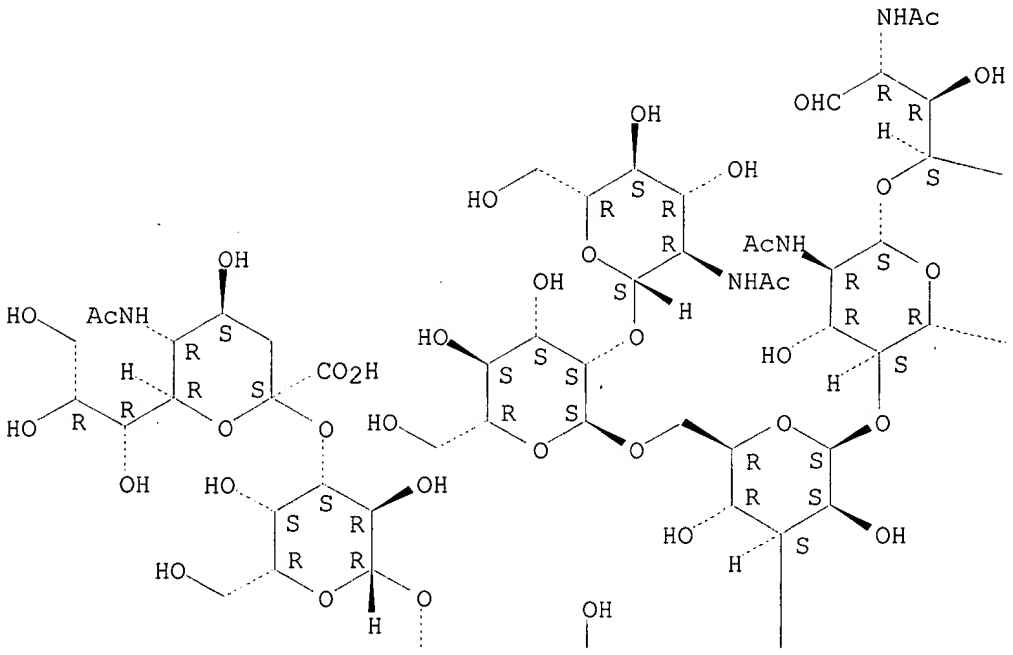
1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 124:169248

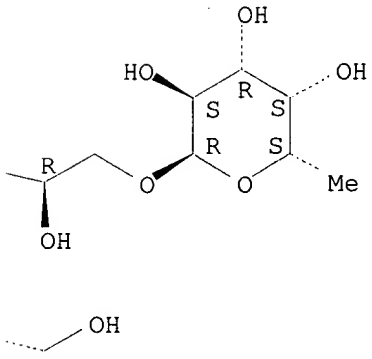
L89 ANSWER 3 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 162715-13-1 REGISTRY
CN D-Glucose, O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-.beta.-D-galactopyranosyl-(1.fwdarw.3)]-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)]-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
FS STEREOSEARCH
MF C92 H151 N7 O66
SR CA
LC STN Files: CA, CAPLUS

Absolute stereochemistry.

PAGE 1-A



PAGE 1-B



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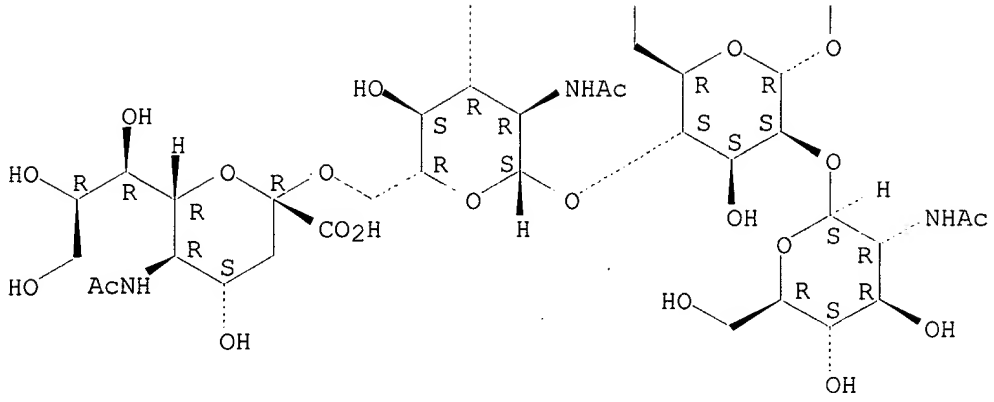


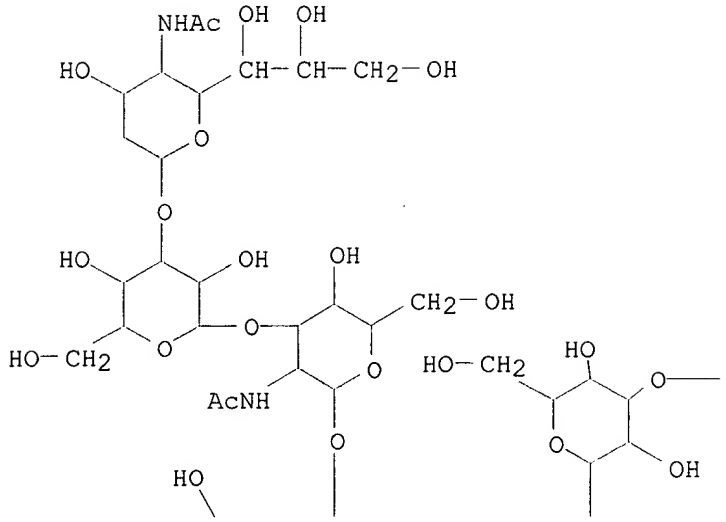
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

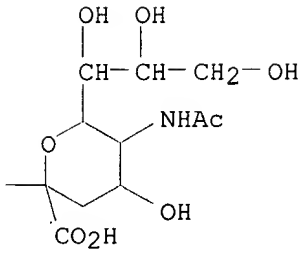
REFERENCE 1: 122:259079

L89 ANSWER 4 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144185-75-1 REGISTRY
CN D-Glucose, O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.6)]-O-.alpha.-D-mannopyranosyl-(1.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)]-.alpha.-D-mannopyranosyl-(1.fwdarw.3)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C122 H201 N9 O87
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

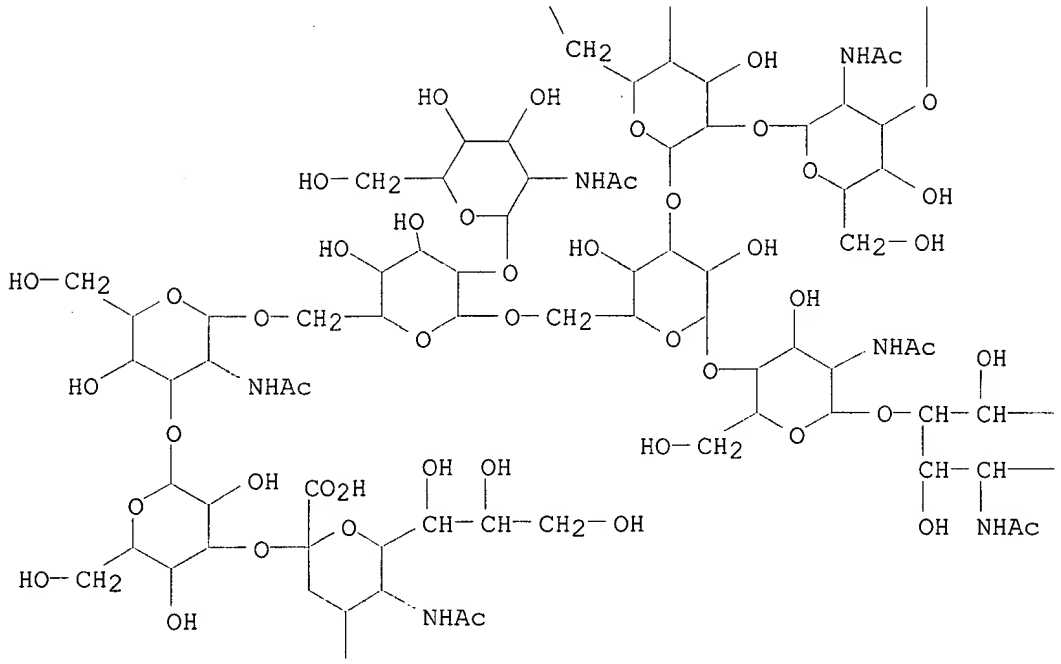
PAGE 1-A



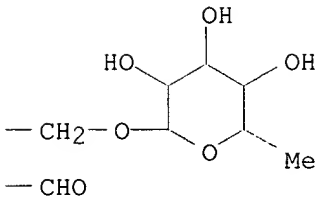
PAGE 1-B



PAGE 2-A



PAGE 2-B



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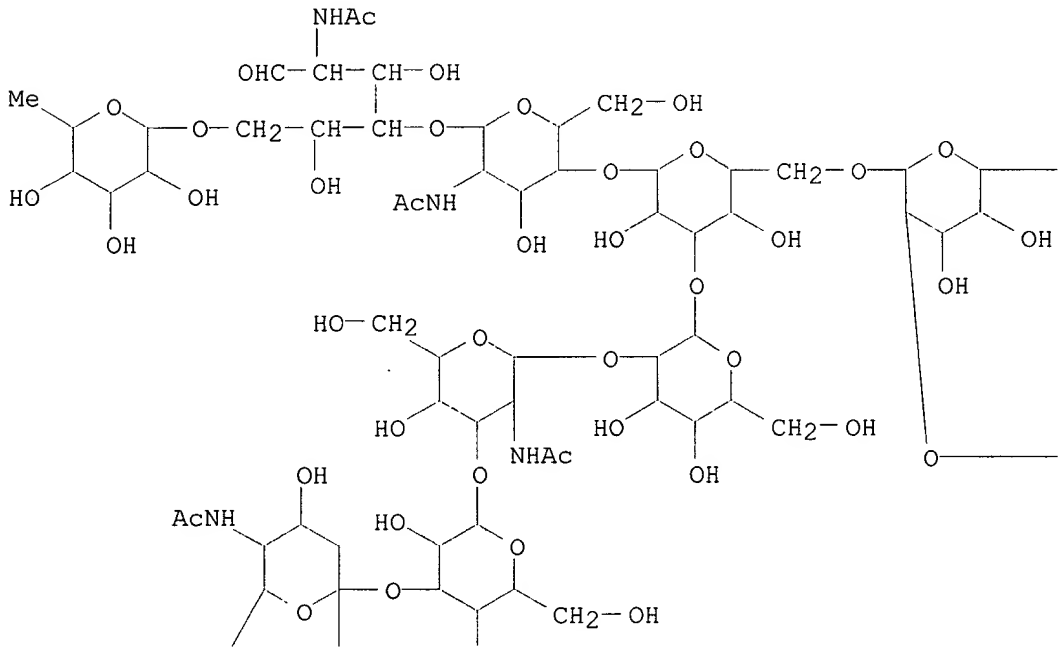
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

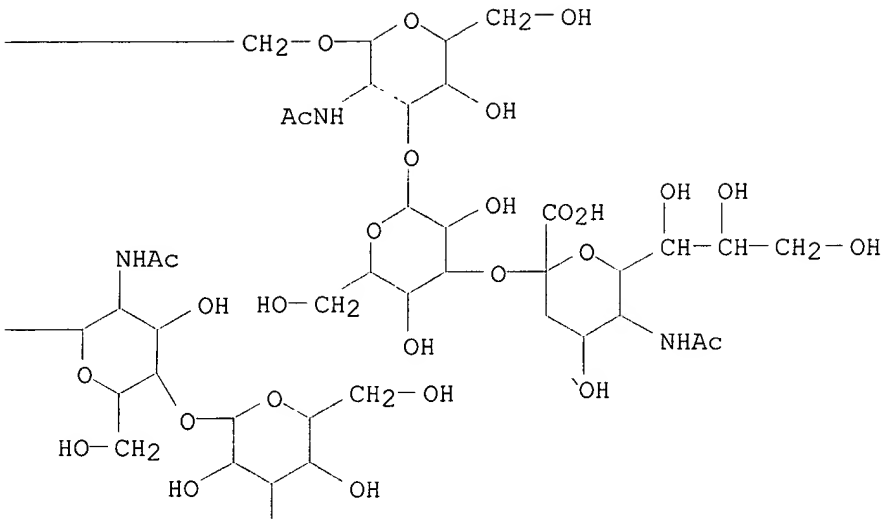
REFERENCE 1: 117:207864

L89 ANSWER 5 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144185-74-0 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.4)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)]-O-.alpha.-D-mannopyranosyl-(1.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.3)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C115 H188 N8 O84
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

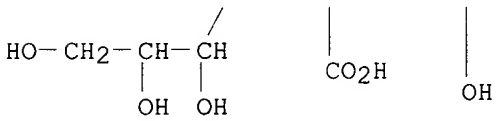
PAGE 1-A



PAGE 1-B



PAGE 2-A



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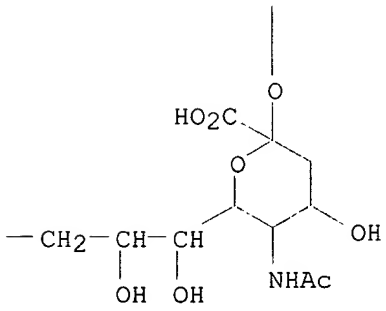
PAGE 2-B



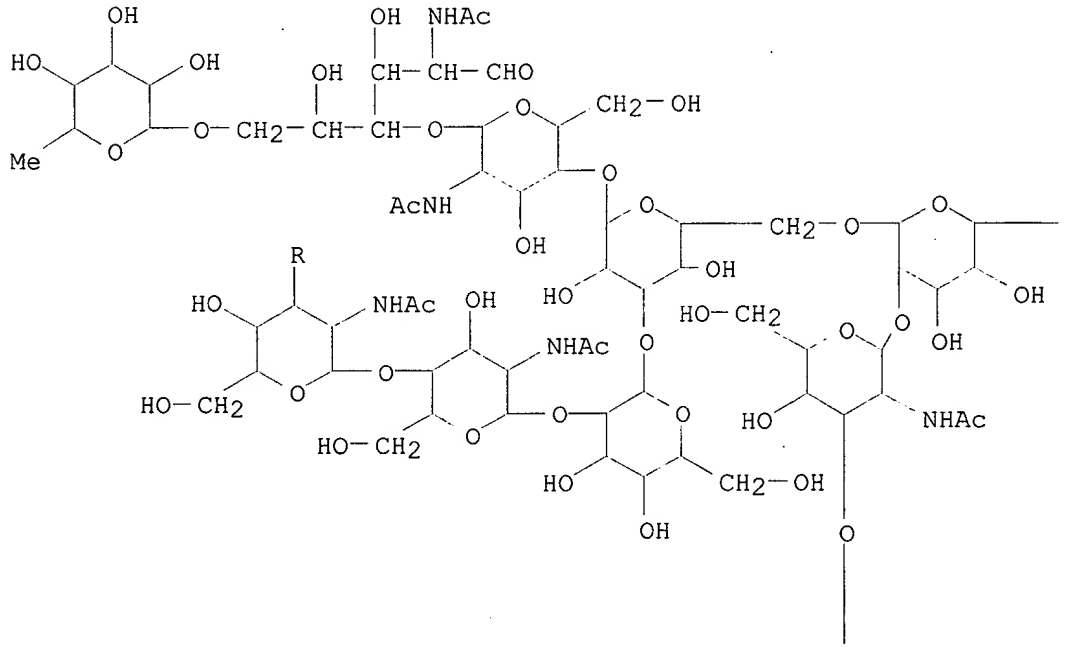
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

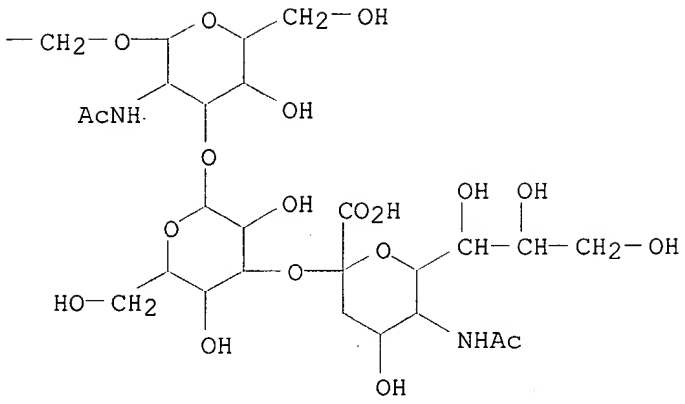
REFERENCE 1: 117:207864

L89 ANSWER 6 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144185-73-9 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-galactopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.6)]-.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C117 H191 N9 O84
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

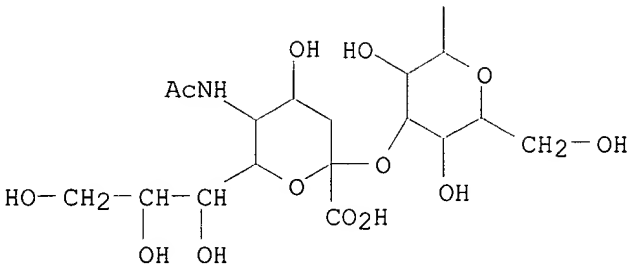
PAGE 1-A

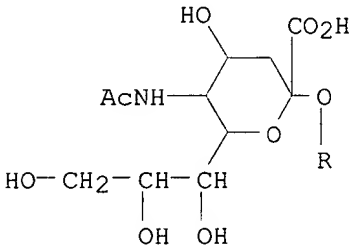


PAGE 1-B



PAGE 2-A





PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 117:207864

L89 ANSWER 7 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144185-69-3 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-

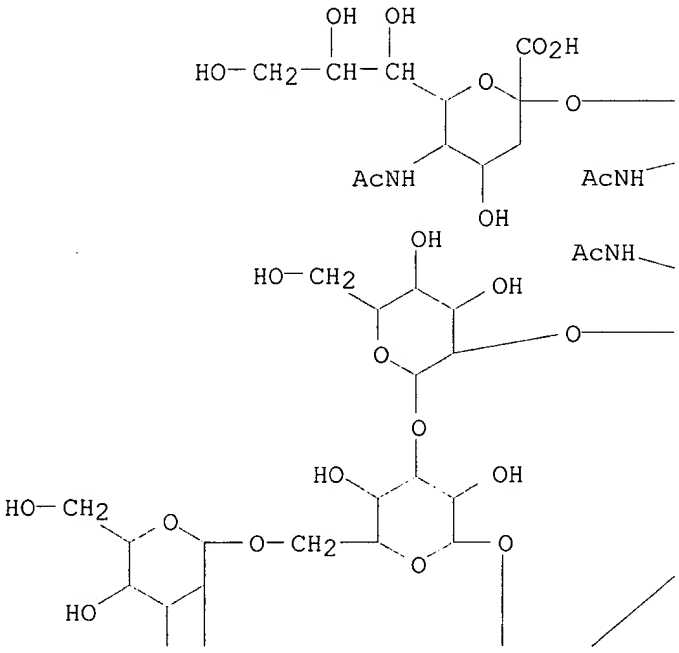
(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)

MF C92 H151 N7 O66

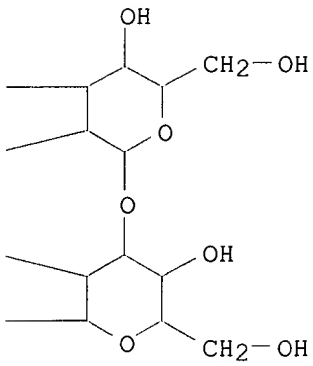
SR CA

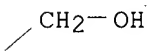
LC STN Files: CA, CAPLUS, TOXCENTER

PAGE 1-A



PAGE 1-B





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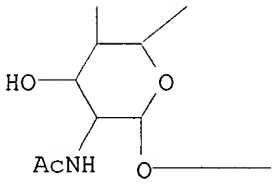
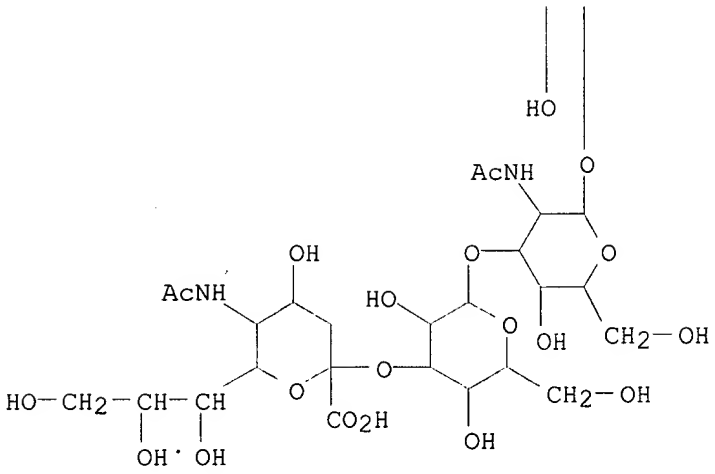
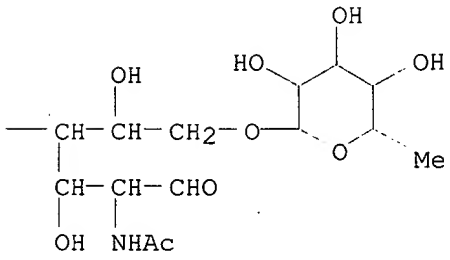




PAGE 2-B



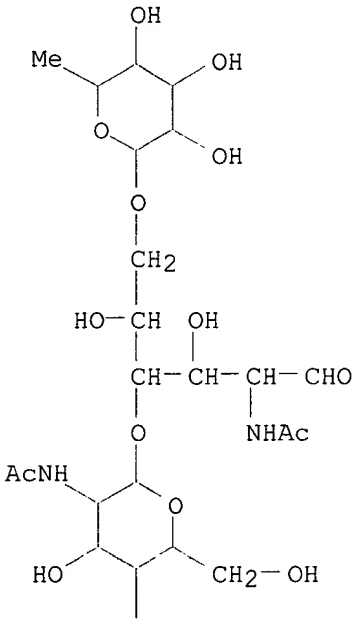
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

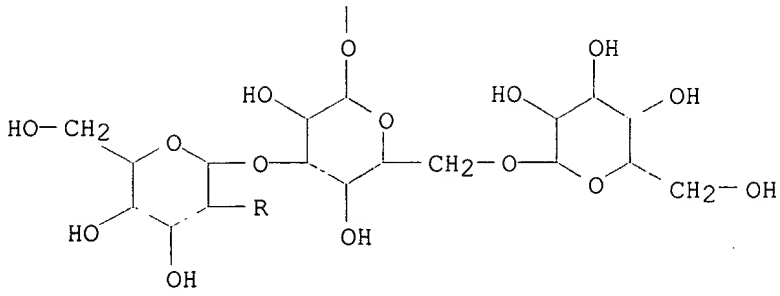
REFERENCE 1: 117:207864

L89 ANSWER 8 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144185-68-2 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C65 H108 N4 O48
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

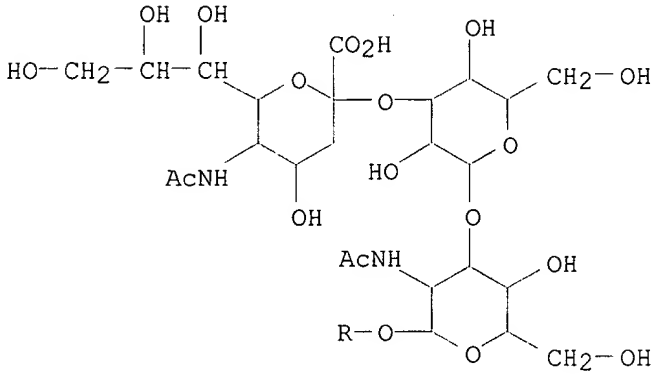
PAGE 1-A



PAGE 2-A



PAGE 3-A



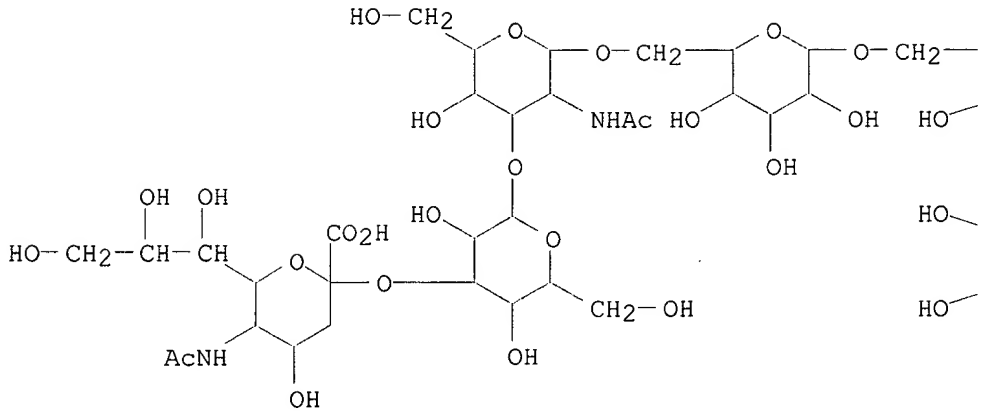
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

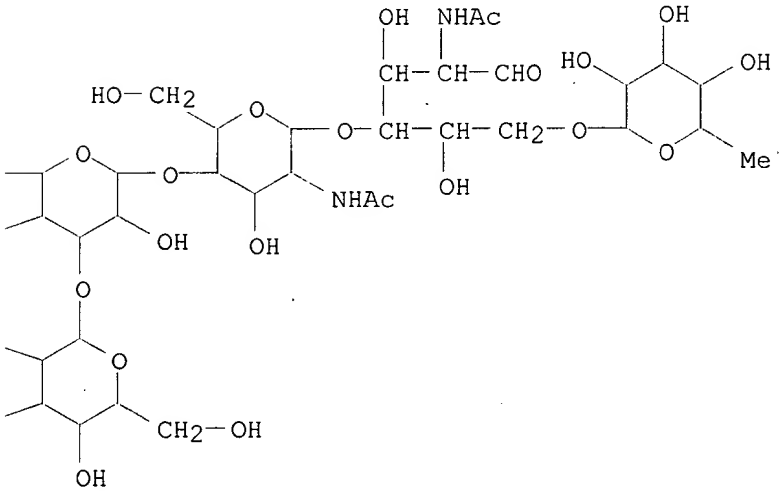
REFERENCE 1: 117:207864

L89 ANSWER 9 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144185-67-1 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.6)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.6)-O-[.alpha.-D-mannopyranosyl-(1.fwdarw.3)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C65 H108 N4 O48
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

PAGE 1-A



PAGE 1-B



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

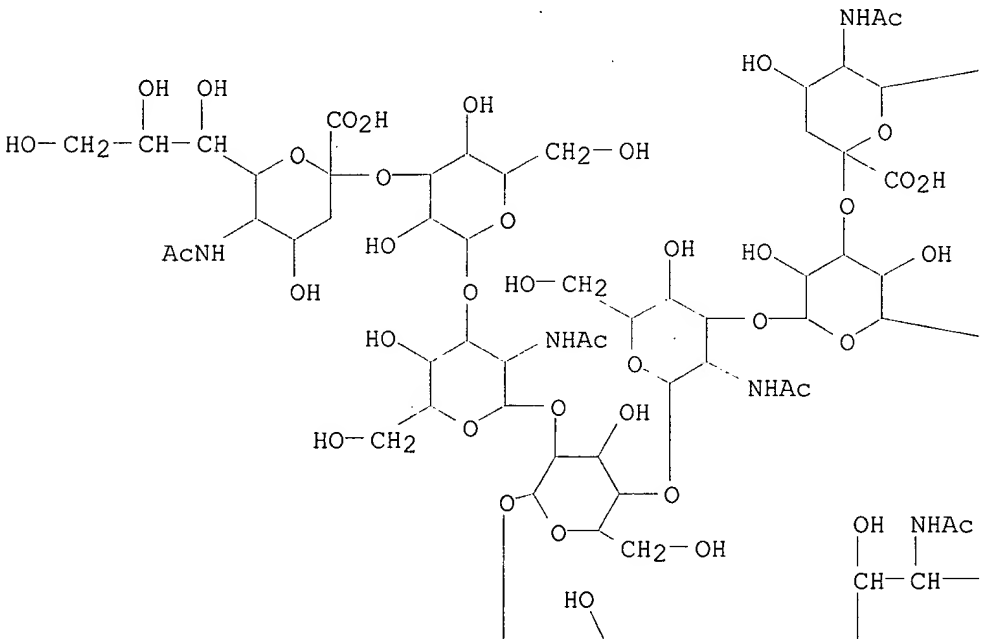
REFERENCE 1: 117:207864

L89 ANSWER 10 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144161-60-4 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)]-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-

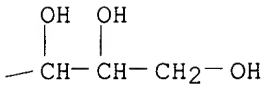
galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)

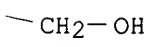
MF C115 H188 N8 O84
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

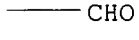
PAGE 1-A



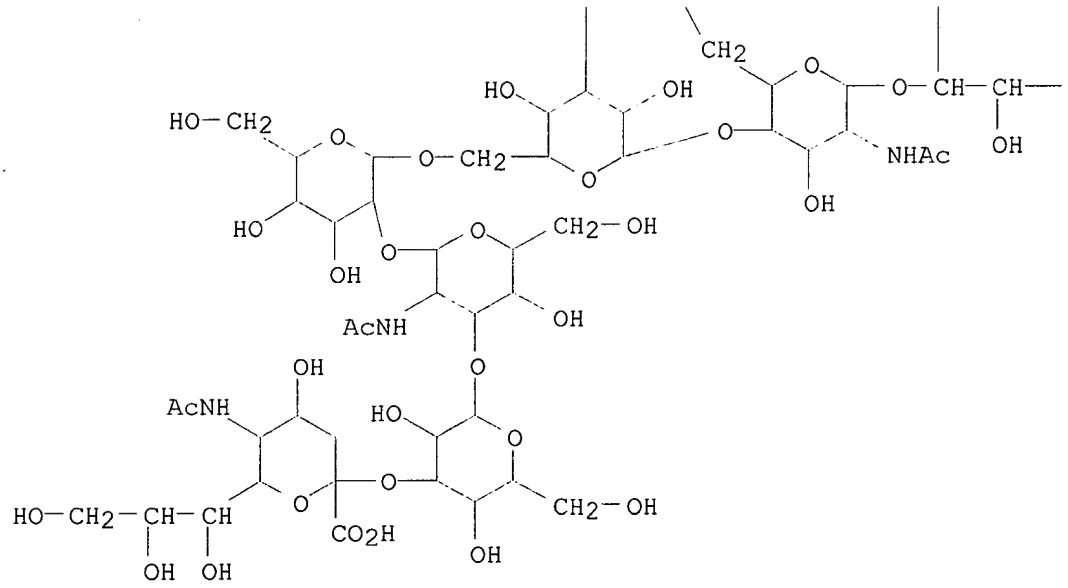
PAGE 1-B



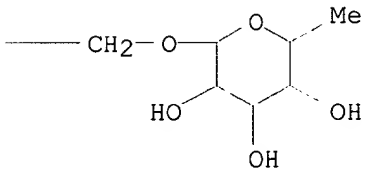




PAGE 2-A



PAGE 2-B



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

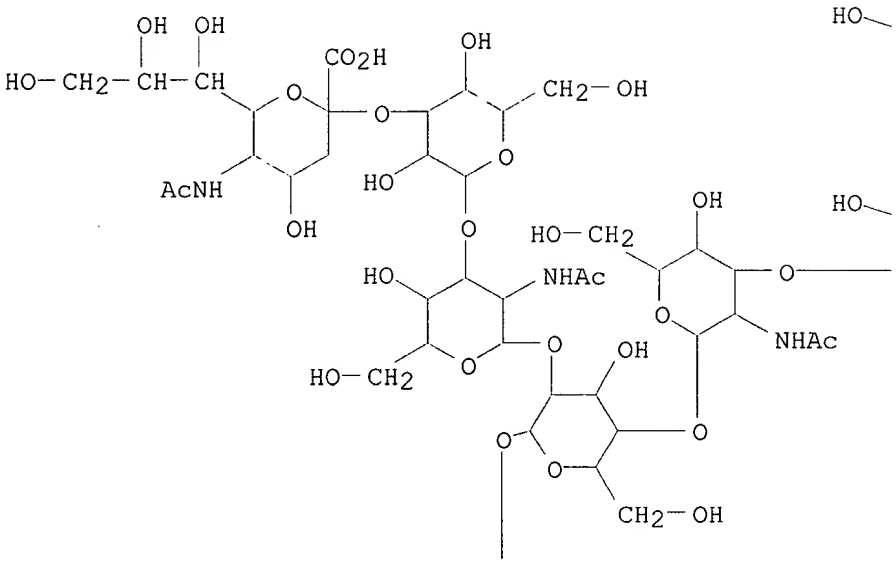
1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 117:207864

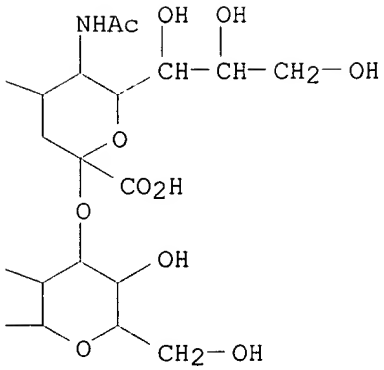
L89 ANSWER 11 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144161-59-1 REGISTRY
CN D-Glucose, O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)]-O-.alpha.-D-mannopyranosyl-(1.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)]-.alpha.-D-mannopyranosyl-(1.fwdarw.3)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C123 H201 N9 O89

SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

PAGE 1-A



PAGE 1-B



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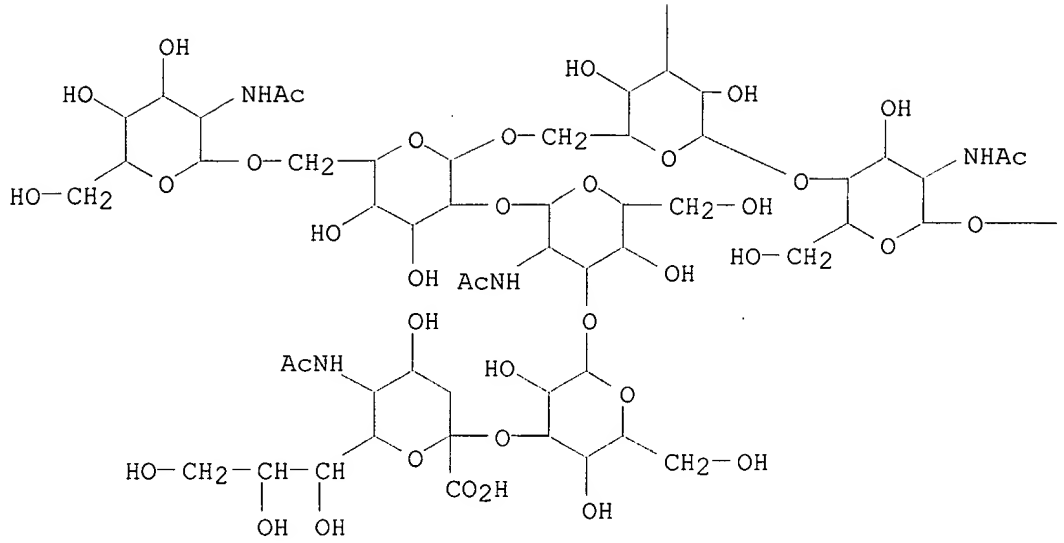
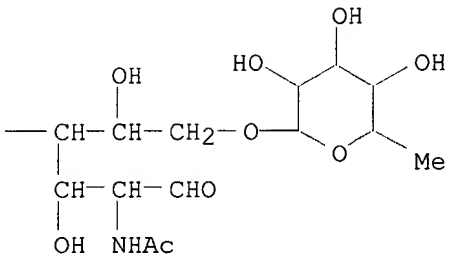


PAGE 2-B



PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

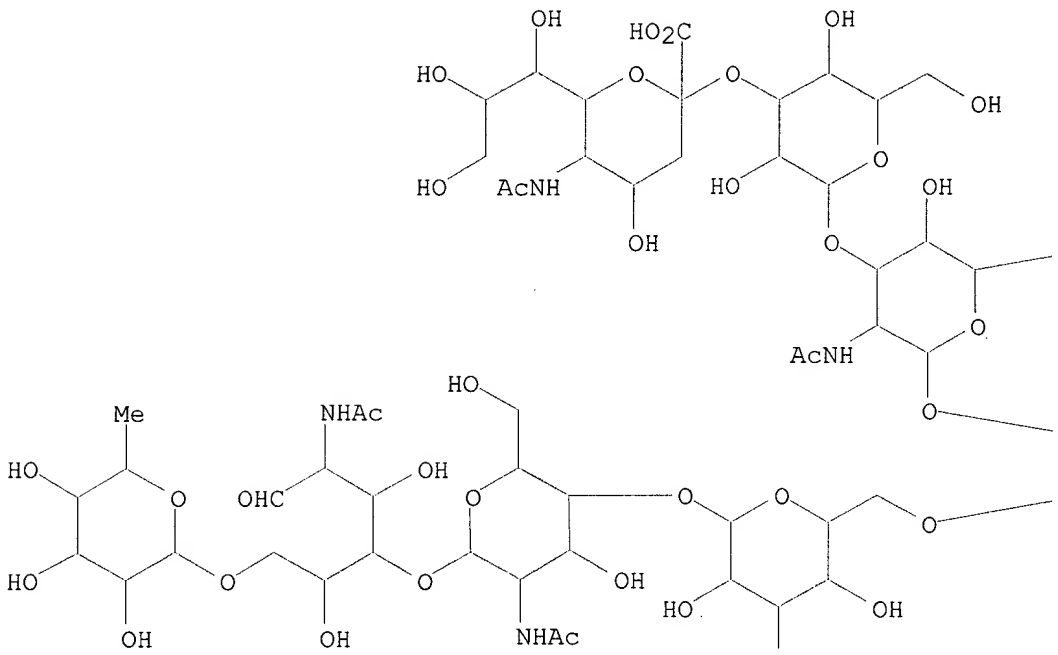
1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 117:207864

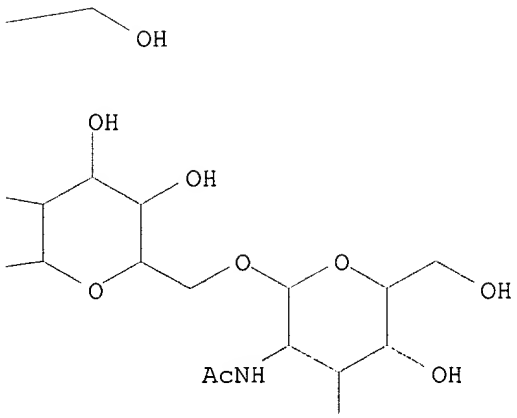
L89 ANSWER 12 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144161-58-0 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.6)]-O-.alpha.-D-mannopyranosyl-(1.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-.alpha.-D-mannopyranosyl-(1.fwdarw.3)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C115 H188 N8 O84

SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

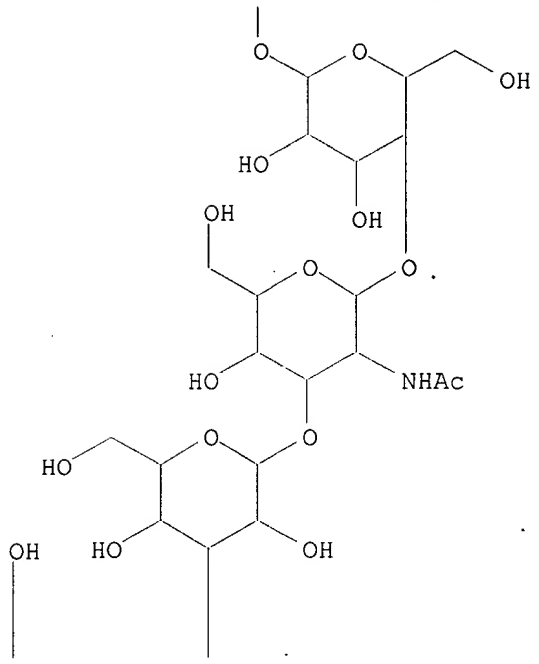
PAGE 1-A



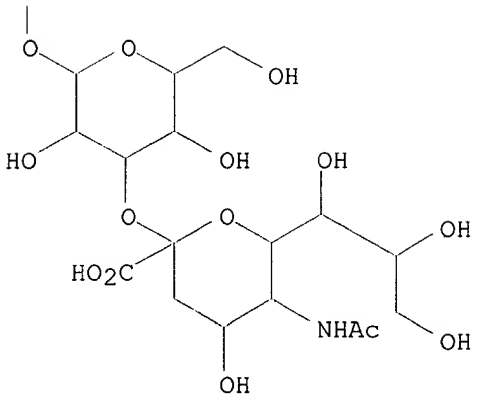
PAGE 1-B



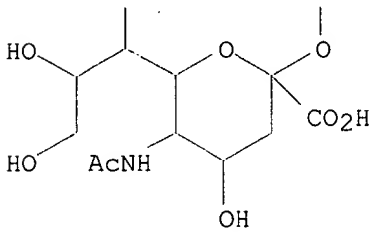
PAGE 2-A



PAGE 2-B



PAGE 3-A



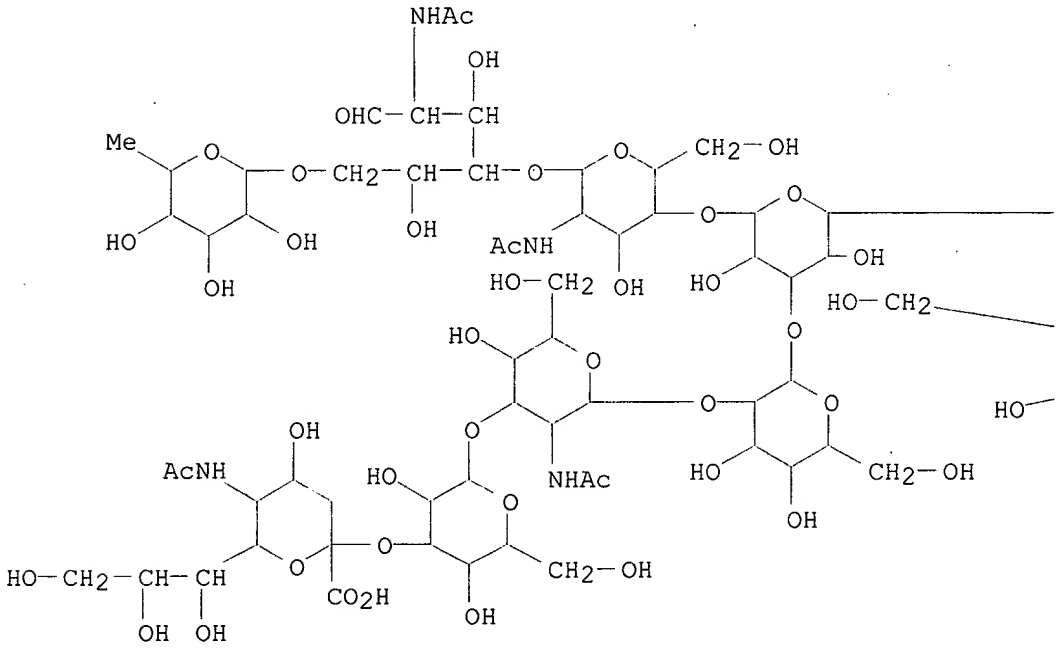
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 117:207864

L89 ANSWER 13 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144161-57-9 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.6)]-O-.alpha.-D-mannopyranosyl-(1.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.3)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C115 H188 N8 O84
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

PAGE 1-A



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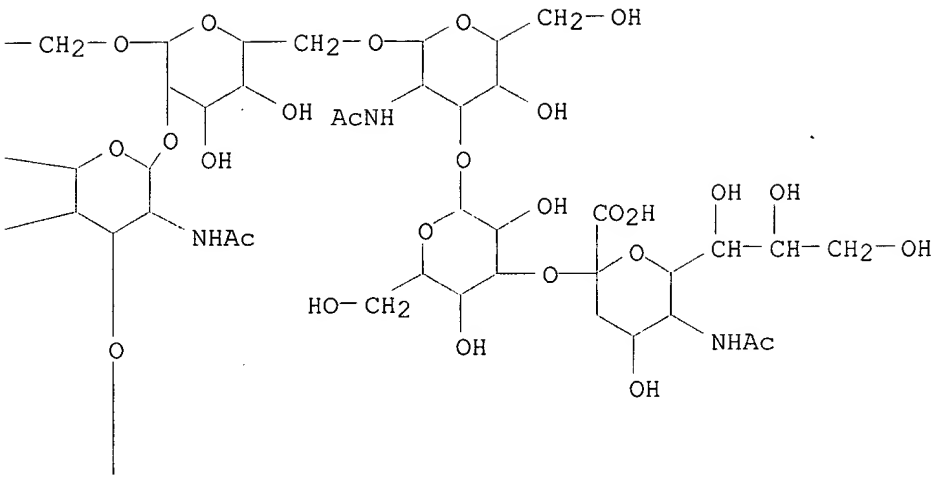
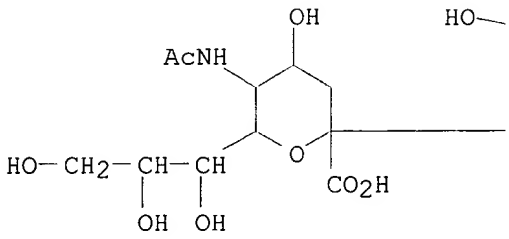
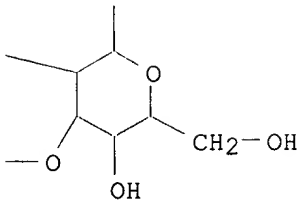


PAGE 2-A



PAGE 2-B



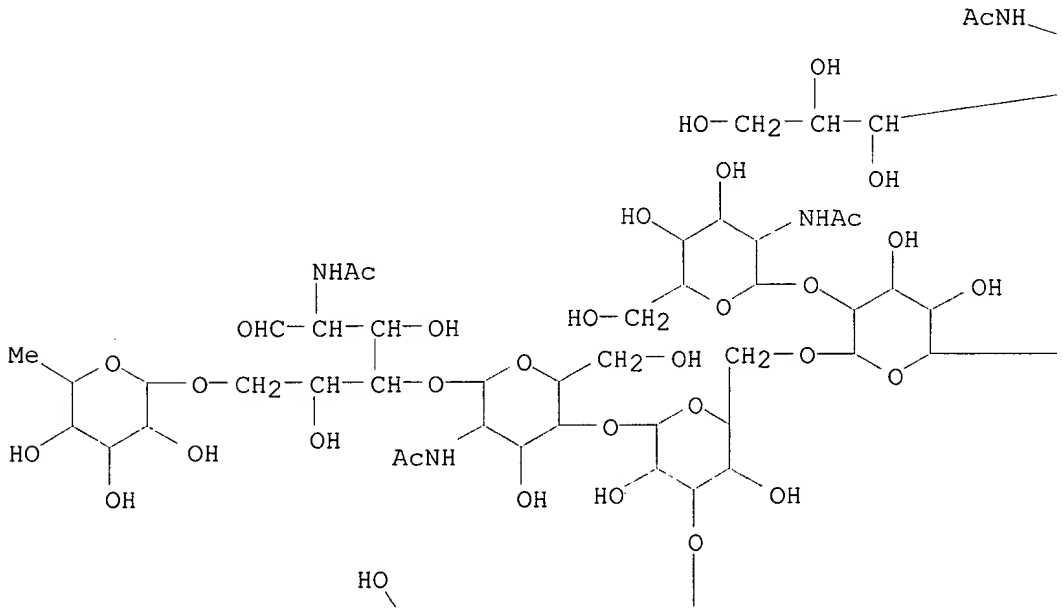
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

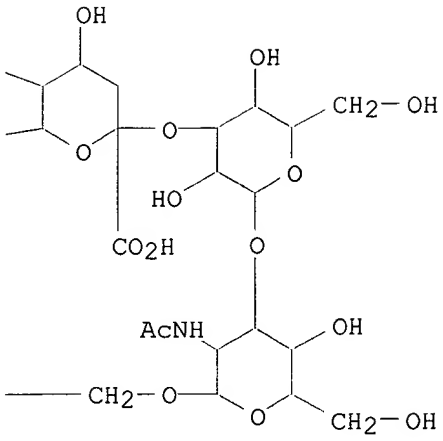
REFERENCE 1: 117:207864

L89 ANSWER 14 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144161-56-8 REGISTRY
CN D-Glucose, O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.6)]-O-.alpha.-D-mannopyranosyl-(1.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.3)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C98 H161 N7 O71
SR CA
LC STN Files: CA, CAPLUS, TOXCENTER

PAGE 1-A



PAGE 1-B



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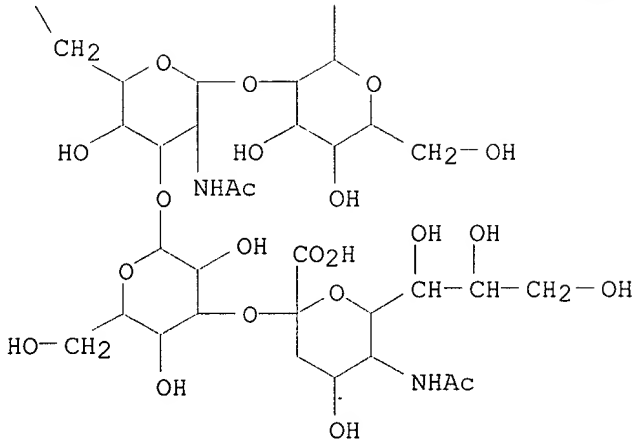


PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

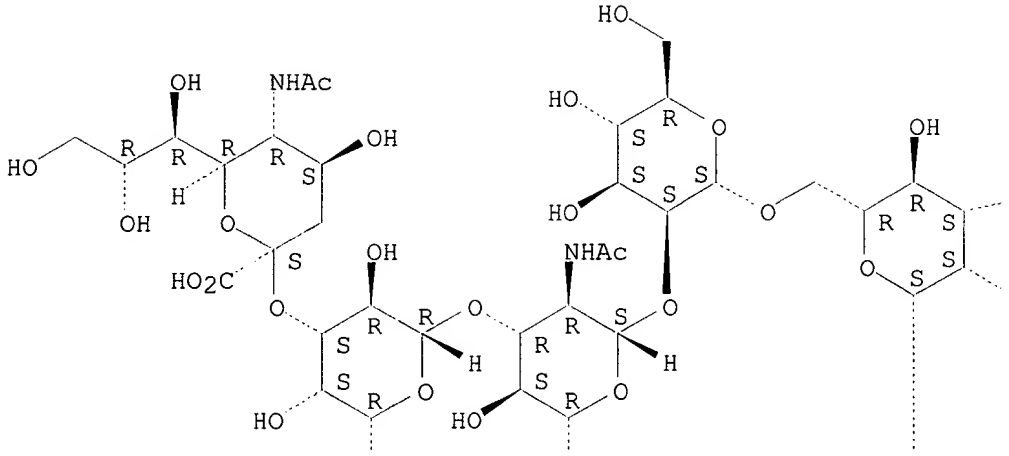
REFERENCE 1: 117:207864

L89 ANSWER 15 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 144161-29-5 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
FS STEREOSEARCH
MF C90 H148 N6 O66
SR CA

LC STN Files: CA, CAPLUS, TOXCENTER

Absolute stereochemistry.

PAGE 1-A



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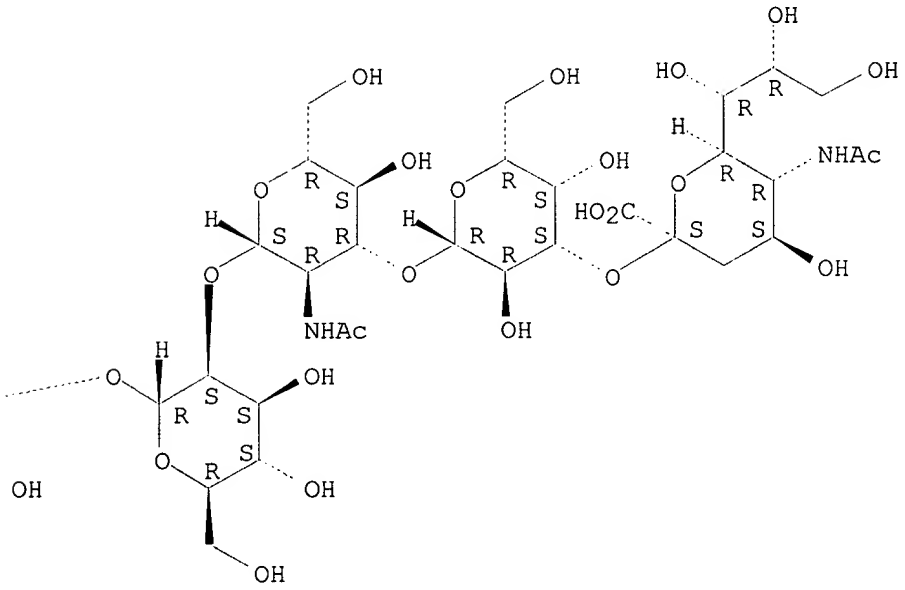

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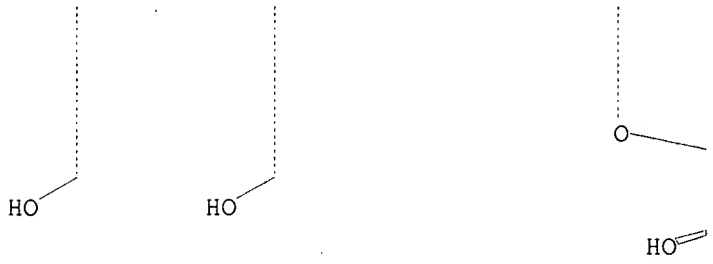

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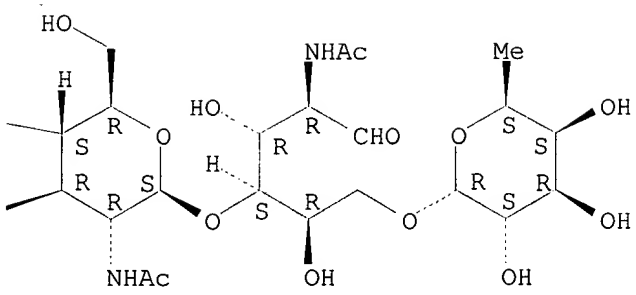


PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

3 REFERENCES IN FILE CA (1962 TO DATE)
3 REFERENCES IN FILE CAPLUS (1962 TO DATE)

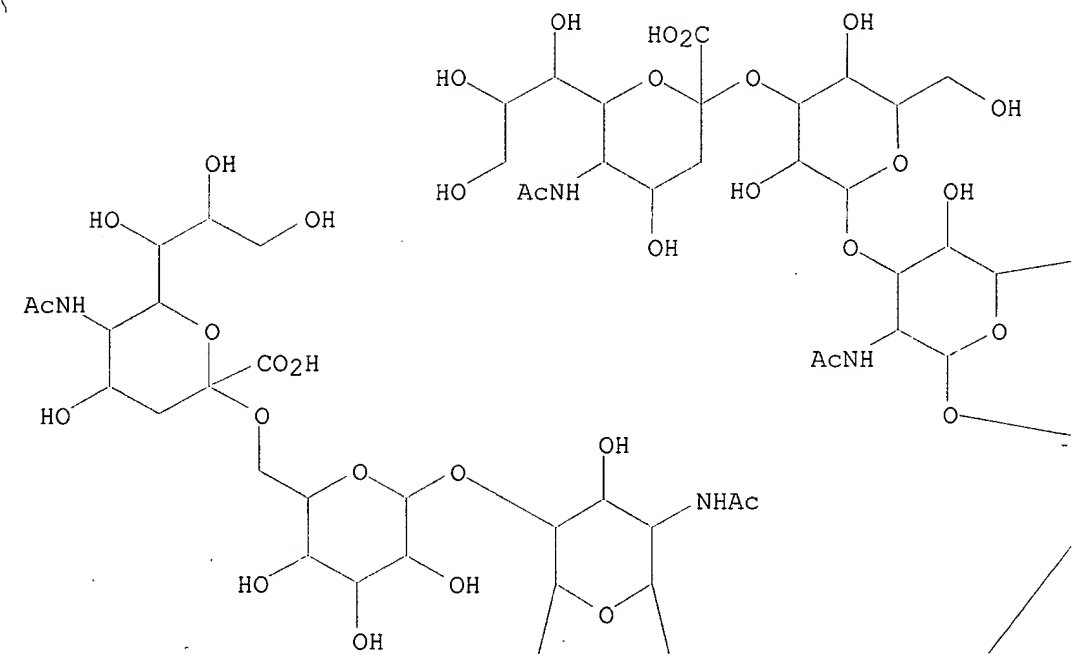
REFERENCE 1: 135:253672

REFERENCE 2: 124:169248

REFERENCE 3: 117:207864

L89 ANSWER 16 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 135376-92-0 REGISTRY
CN D-Glucose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-.beta.-D-galactopyranosyl-(1.fwdarw.3)]-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.6)-O-.beta.-D-galactopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C101 H165 N7 O74
SR CA
LC STN Files: CA, CAPLUS

PAGE 1-A



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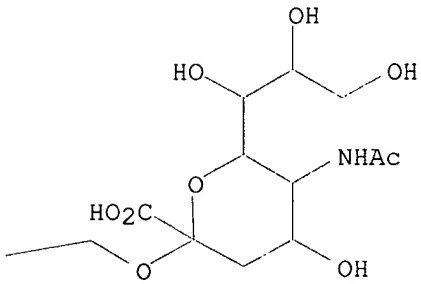

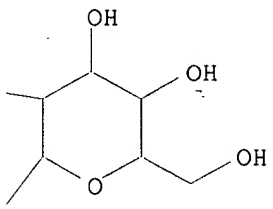

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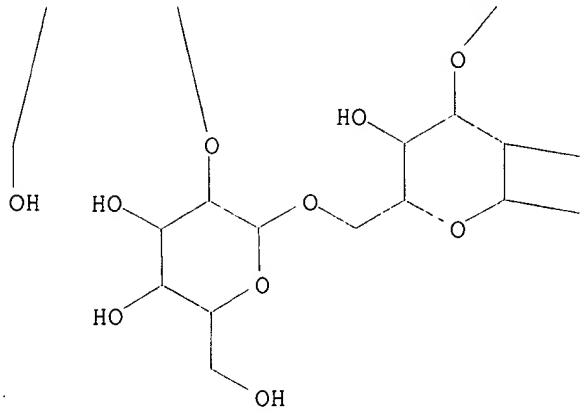

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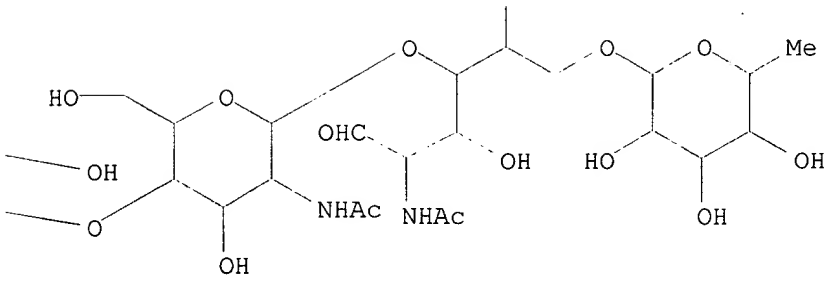

PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 115:90311

L89 ANSWER 17 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 133179-78-9 REGISTRY
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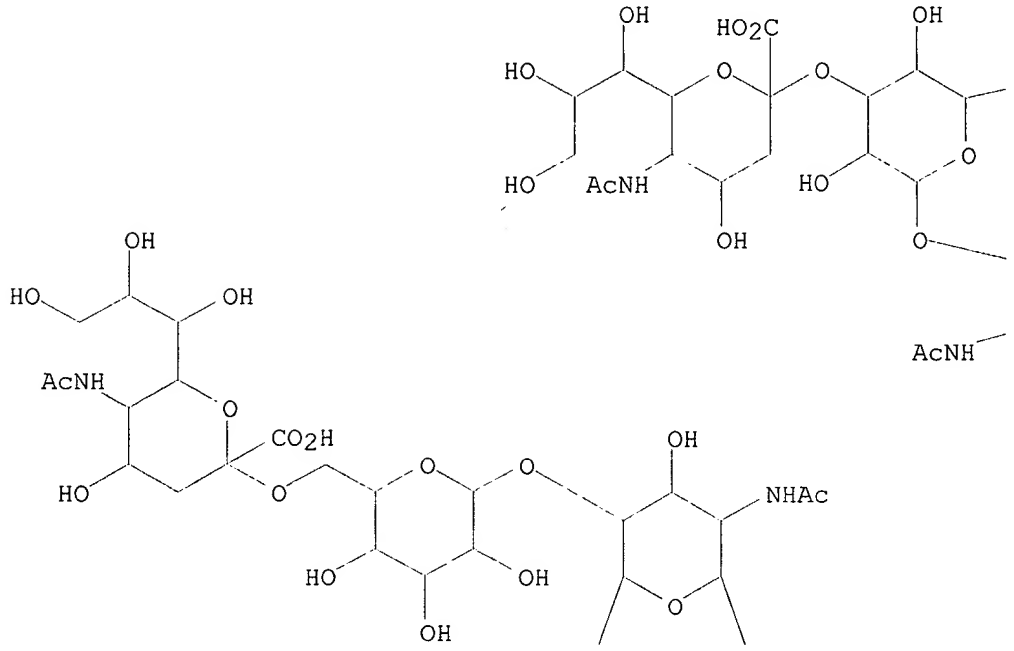
MF C101 H165 N7 O74
SR CA
LC STN Files: CA, CAPLUS

PAGE 1-A



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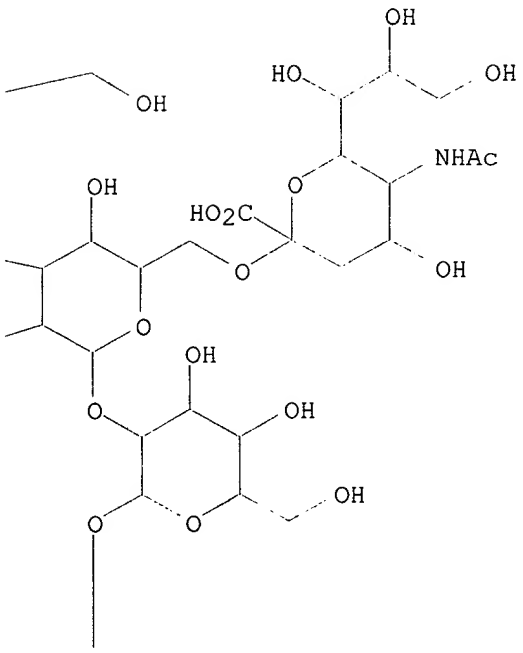

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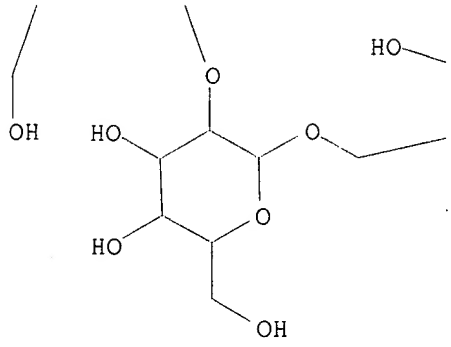

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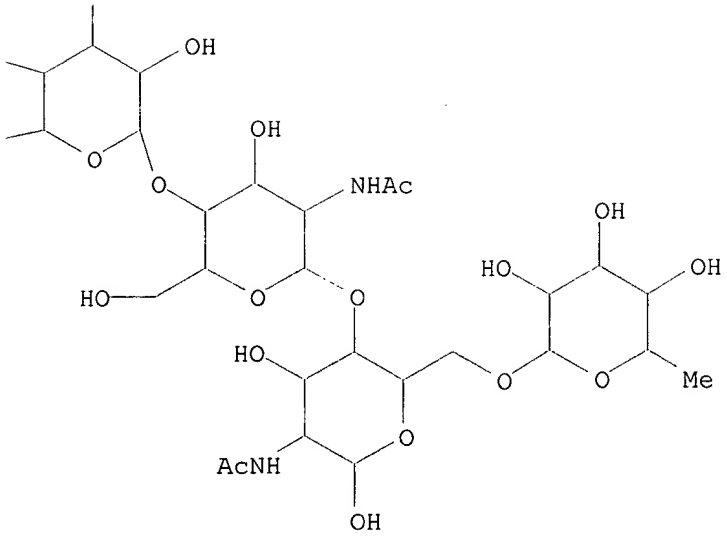


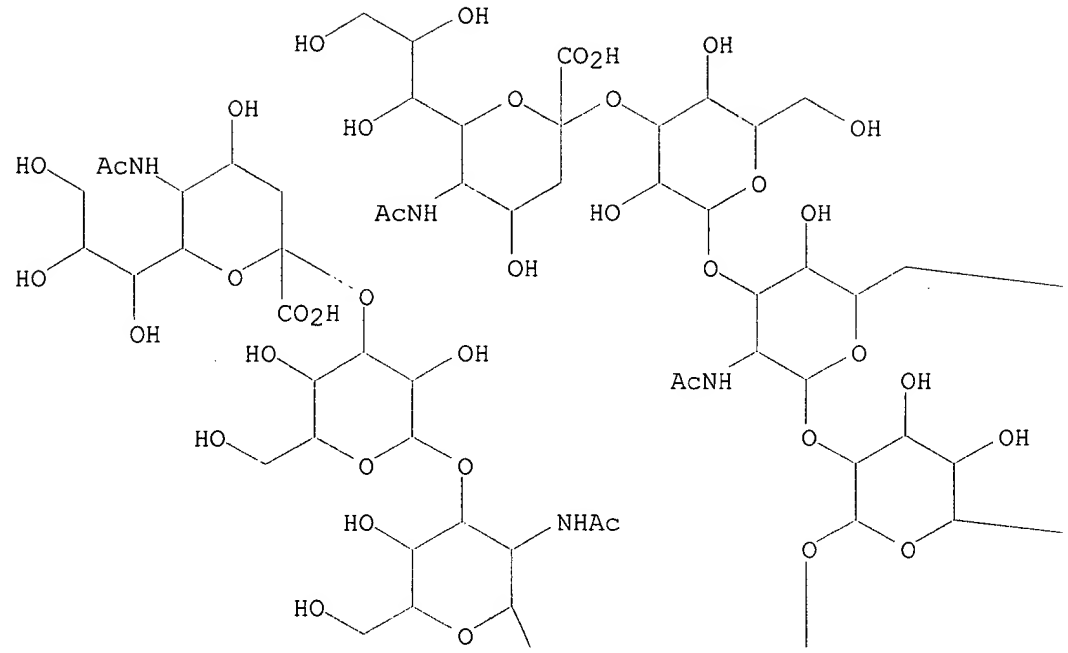
PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT

1 REFERENCES IN FILE CA (1962 TO DATE)
1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE 1: 114:159396

L89 ANSWER 18 OF 18 REGISTRY COPYRIGHT 2002 ACS
RN 133157-60-5 REGISTRY
CN .beta.-D-Glucopyranose, O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.6)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-.beta.-D-galactopyranosyl-(1.fwdarw.3)]-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-O-.alpha.-D-mannopyranosyl-(1.fwdarw.3)-O-[O-(N-acetyl-.alpha.-neuraminosyl)-(2.fwdarw.3)-O-.beta.-D-galactopyranosyl-(1.fwdarw.3)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.2)-.alpha.-D-mannopyranosyl-(1.fwdarw.6)]-O-.beta.-D-mannopyranosyl-(1.fwdarw.4)-O-2-(acetylamino)-2-deoxy-.beta.-D-glucopyranosyl-(1.fwdarw.4)-O-[6-deoxy-.alpha.-L-galactopyranosyl-(1.fwdarw.6)]-2-(acetylamino)-2-deoxy- (9CI) (CA INDEX NAME)
MF C101 H165 N7 O74
SR CA
LC STN Files: CA, CAPLUS

PAGE 1-A



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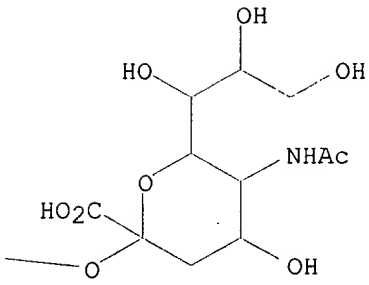

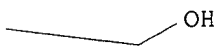

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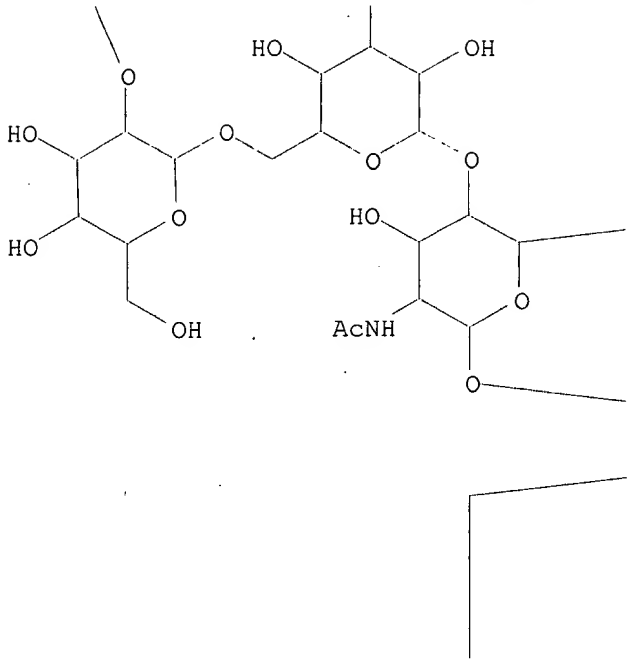

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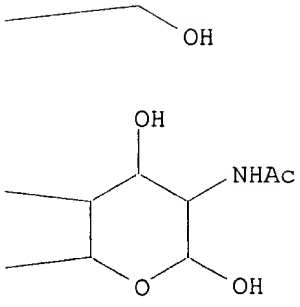

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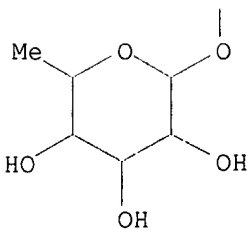

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**PROPERTY DATA AVAILABLE IN THE 'PROP' FORMAT**

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               1 REFERENCES IN FILE CAPLUS (1962 TO DATE)

REFERENCE   1:  114:159396


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                SET COST OFF

     FILE 'REGISTRY' ENTERED AT 13:36:51 ON 08 NOV 2002
                E ERYTHROPOIETIN/CN
L1             1 S E3
                E ERYTHROPOIETIN
L2          1166 S E3 NOT L1
L3             0 S L2 AND OC5/ES
L4             0 S L2 AND PMS/CI
                SEL RN L1
L5             6 S E1/CRN
L6          1166 S L2,L5

     FILE 'HCAPLUS' ENTERED AT 13:38:18 ON 08 NOV 2002
L7          7105 S L1
L8           359 S L6
L9          9347 S ERYTHROPOIETIN
L10         4784 S EPOETIN OR EPO
L11        11366 S L7-L10
                E BURG J/AU
L12           67 S E3-E11,E24
                E BUERG J/AU
                E BEURG J/AU
                E SELLINGER K/AU
L13           10 S E4,E5
                E HASELBECK A/AU
L14           27 S E3,E4
                E HAESELBECK A/AU
                E HEASELBECK A/AU
                E KOLL H/AU
L15           27 S E3,E4,E6-E8
                E KOELL H/AU
L16            1 S E4
                E KEOLL H/AU
L17           14 S L11 AND L12-L16
                E DE97-19753681/AP,PRN
L18            1 S E3,E4
                E DE98-19813415/AP,PRN
                E WO98-EP7876/AP,PRN
L19            1 S E3,E4
                E D HIS
                E EP98-113415/AP,PRN
L20            1 S E4
L21            1 S L12-L17 AND L18-L20
L22           14 S L17,L21
L23            3 S L12 AND L13-L17
L24            1 S L13 AND L14-L17
L25            0 S L15 AND L16
L26            3 S L23,L24
L27           14 S L22-L26
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```
L28         170 S L11 (L) GLYCOSYLAT?
                E GLYCOSYLATION/CT
                E E4+ALL
L29        1817 S E2
                E GLYCOSYLATION/CT
                E E3+ALL
L30       18186 S E4,E3+NT
                E E41+ALL
L31         605 S E4,E3+NT
L32          99 S L11 AND L29-L31
L33         204 S L28,L32
L34          11 S L33 AND (ACETYLLACTOSAMINE OR ACETYL(L)LACTOSAMINE)
L35          19 S L33 AND (?ACETYLLACTOSAMIN? OR ?ACETYL?(L)?LACTOSAMIN?)
L36          19 S L34,L35

     FILE 'REGISTRY' ENTERED AT 13:51:10 ON 08 NOV 2002
L37           1 S 32181-59-2
L38         238 S C14H25NO11/MF
L39         233 S L38 AND OC5/ES
L40         192 S L39 AND ACETYLAMINO
L41          43 S L40 AND GLUCOSE
L42          17 S L41 AND GALACTO?
L43           5 S L42 AND 4 NOT (T/ELS OR 13C# OR 6)
L44           4 S L43 NOT IDS/CI
L45           3 S L44 NOT 92762-44-2
L46          12 S L42 NOT L43
                SEL RN 2 3
L47           2 S E1-E2
L48           5 S L45,L47
                SEL RN
L49           5 S E3-E7/CRN

     FILE 'HCAPLUS' ENTERED AT 13:57:48 ON 08 NOV 2002
L50         760 S L48 OR L49
L51           8 S L50 AND L11
L52           3 S L36 AND L51
L53           5 S L51 NOT L52
                SEL DN AN 3 4 5
L54           3 S E8-E16 AND L53
L55           6 S L52,L54
L56          16 S L36 NOT L55
L57          22 S L55,L56
L58          36 S L27,L57
L59          21 S L58 AND ?LACTOSAMIN?
L60          36 S L58,L59

     FILE 'REGISTRY' ENTERED AT 14:11:20 ON 08 NOV 2002
L61             STR
L62          50 S L61
                STR L61
L64        9510 S L63 FUL
                SAV L64 AUDET555/A
L65         121 S L64 AND PMS/CI
L66        9387 S L64 NOT L49,L65
L67             STR
L68          50 S L67 SAM SUB=L66
L69        1402 S L67 FUL SUB=L66
                SAV L69 AUDET555A/A
L70             STR
L71          17 S L70 SAM SUB=L69
L72         373 S L70 FUL SUB=L69
                SAV L72 AUDET555B/A
L73        1029 S L69 NOT L72
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L74          41 S L73 AND IDS/CI
L75         988 S L73 NOT L74
L76          40 S L74 NOT SPIRO

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L77         768 S L75 OR L76
L78           3 S L77 AND L11
L79          39 S L60,L78

     FILE 'REGISTRY' ENTERED AT 14:32:52 ON 08 NOV 2002
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L81             STR L80
L82         511 S L81 FUL SUB=L73
                SAV L82 AUDET555C/A
L83             STR
L84           0 S L83 SAM SUB=L82
L85           1 S L83 FUL SUB=L82
                SAV L85 AUDET555D/A
L86             STR L67
L87          48 S L86 FUL SUB=L82
                SAV L87 AUDET555E/A
L88             STR L86
L89          18 S L88 FUL SUB=L87
                SAV L89 AUDET555F/A
L90           0 S L89 AND NR>=21

     FILE 'HCAPLUS' ENTERED AT 15:15:07 ON 08 NOV 2002
L91           7 S L89 OR L85

     FILE 'REGISTRY' ENTERED AT 15:15:32 ON 08 NOV 2002
L92           3 S L48 NOT L47

=> fil hcaplus
FILE 'HCAPLUS' ENTERED AT 15:18:04 ON 08 NOV 2002
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FILE COVERS 1907 - 8 Nov 2002  VOL 137 ISS 20
FILE LAST UPDATED: 7 Nov 2002  (20021107/ED)

 This file contains CAS Registry Numbers for easy and accurate
 substance identification.

CAS roles have been modified effective December 16, 2001. Please
check your SDI profiles to see if they need to be revised. For
information on CAS roles, enter HELP ROLES at an arrow prompt or use
the CAS Roles thesaurus (/RL field) in this file.

=> d l91 all tot

L91  ANSWER 1 OF 7  HCAPLUS  COPYRIGHT 2002 ACS
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AN 2001:543015 HCAPLUS
DN 135:253672
TI Structure and characterization of the glycan moiety of L-amino-acid oxidase from the Malayan pit viper Calloselasma rhodostoma
AU Geyer, Armin; Fitzpatrick, Teresa B.; Pawelek, Peter D.; Kitzing, Karina; Vrielink, Alice; Ghisla, Sandro; Macheroux, Peter
CS Section of Natural Sciences, Universitat Konstanz, Germany
SO European Journal of Biochemistry (2001), 268(14), 4044-4053
CODEN: EJBCAI; ISSN: 0014-2956
PB Blackwell Science Ltd.
DT Journal
LA English
CC 7-5 (Enzymes)
Section cross-reference(s): 6
AB Ophidian L-amino-acid oxidase (L-amino-acid oxygen:oxidoreductase, deaminating, EC 1.4.3.2) is found in the venom of many poisonous snakes (crotalids, elapids and viperids). This FAD-dependent glycoprotein has been studied from several snake species (e.g. Crotalus adamanteus, Crotalus atrox and Calloselasma rhodostoma) in detail with regard to the biochem. and enzymic properties. The nature of glycosylation, however, as well as the chem. structure(s) of the attached oligosaccharide(s) are unknown. In view of the putative involvement of the glycan moiety in the biol. effects of ophidian L-amino-acid oxidase, notably the apoptotic activity of the enzyme, structural knowledge is needed to evaluate its exact function. In this study we report on the glycosylation of L-amino-acid oxidase from the venom of the Malayan pit viper (Calloselasma rhodostoma). Its glycosylation is remarkably homogeneous with the major oligosaccharide accounting for approx. 90% of the total sugar content. Based on detailed anal. of the isolated oligosaccharide by 2D NMR spectroscopies and MALDI-TOF mass spectrometry the glycan is identified as a bis-sialylated, biantennary, core-fucosylated dodecasaccharide. The biol. significance of this finding is discussed in light of the biol. activities of the enzyme.
ST amino acid oxidase Calloselasma glycan structure
IT Calloselasma rhodostoma
(Asn-linked glycan in L-amino-acid oxidase from Calloselasma rhodostoma is bis-sialylated, biantennary, core-fucosylated dodecasccharide)
IT Oligosaccharides, properties
RL: PRP (Properties)
(N-linked; Asn-linked glycan in L-amino-acid oxidase from Calloselasma rhodostoma is bis-sialylated, biantennary, core-fucosylated dodecasccharide)
IT Venoms
(snake; Asn-linked glycan in L-amino-acid oxidase from C. rhodostoma venom is bis-sialylated, biantennary, core-fucosylated dodecasccharide)
IT 70-47-3, L-Asparagine, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(Asn-linked glycan in L-amino-acid oxidase from Calloselasma rhodostoma is bis-sialylated, biantennary, core-fucosylated dodecasccharide)
IT 9000-89-9, L-Amino-acid oxidase **144161-29-5**
RL: PRP (Properties)
(Asn-linked glycan in L-amino-acid oxidase from Calloselasma rhodostoma is bis-sialylated, biantennary, core-fucosylated dodecasccharide)
RE.CNT 29 THERE ARE 29 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L91 ANSWER 2 OF 7 HCAPLUS COPYRIGHT 2002 ACS
AN 1996:55015 HCAPLUS
DN 124:169248
TI Structural studies of a major hemorrhagin (rhodostoxin) from the venom of Calloselasma rhodostoma (Malayan pit viper)
AU Chung, Maxey C. M.; Ponnudurai, Gnanajothy; Kataoka, Michihiko; Shimizu, Sakayu; Tan, Nget-Hong
CS Dep. Biochemistry, National Univ. Singapore, Singapore, 0511, Singapore
SO Archives of Biochemistry and Biophysics (1996), 325(2), 199-208
CODEN: ABBIA4; ISSN: 0003-9861
PB Academic
DT Journal
LA English
CC 7-5 (Enzymes)
Section cross-reference(s): 4, 12
AB The complete amino acid sequence, disulfide linkages, glycosylation sites, and carbohydrate structure of rhodostoxin, the major hemorrhagin from Calloselasma rhodostoma (Malayan pit viper), have been detd. This sequence confirmed the deduced amino acid sequence of the putative hemorrhagic protein encoded by the prorhodostomin cDNA of C. rhodostoma. Rhodostoxin contained four disulfide bonds that link Cys19-Cys60, Cys117-Cys198, Cys157-Cys182, and Cys159-Cys165. It is the first four-disulfide proteinase reported among all known venom metalloproteinases, which are either of the two-disulfide or three-disulfide type. Peptide-mapping and dot-blotting expts. showed the presence of two glycoproteides. Subsequent sequencing of these peptides established that the N-glycosylation sites are located at residues 91 and 181 of the amino acid sequence of the matured protein. Mass spectrometric analyses of these glycopeptides showed that they contain an oligosaccharide structure consisting of 4 units of N-acetylglucosamine, 5 units of hexose, 1 unit of fucose, and 2 units of neuraminic acids. The complete carbohydrate structure was then established by 2-D mapping anal. of the pyridylamino-oligosaccharides after hydrazinolysis and pyridylamination of the glycan chains.
ST major hemorrhagin rhodostoxin venom Calloselasma sequence; metalloproteinase major hemorrhagin Calloselasma sequence venom
IT Evolution
(of stake venom metalloproteinases; structural studies of major hemorrhagin (rhodostoxin) from venom of Calloselasma rhodostoma (Malayan pit viper))

IT Oligosaccharides
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(protein structure involving; structural studies of major hemorrhagin (rhodostoxin) from venom of Calloselasma rhodostoma (Malayan pit viper))
IT Calloselasma rhodostoma
Protein sequences
Venoms
(structural studies of major hemorrhagin (rhodostoxin) from venom of Calloselasma rhodostoma (Malayan pit viper))
IT Carbohydrates and Sugars, biological studies
RL: BSU (Biological study, unclassified); BIOL (Biological study)
(structural studies of major hemorrhagin (rhodostoxin) from venom of Calloselasma rhodostoma (Malayan pit viper))
IT Bond
(sulfur-sulfur, structural studies of major hemorrhagin (rhodostoxin) from venom of Calloselasma rhodostoma (Malayan pit viper))
IT 173660-46-3
RL: PRP (Properties)
(amino acid sequence contg. 4 disulfide-bonds; structural studies of major hemorrhagin (rhodostoxin) from venom of Calloselasma rhodostoma (Malayan pit viper))
IT 151581-84-9, Rhodostoxin
RL: PRP (Properties)
(contg. four disulfide; structural studies of major hemorrhagin (rhodostoxin) from venom of Calloselasma rhodostoma (Malayan pit viper))
IT 71654-73-4 111945-06-3 **144161-29-5** 173557-95-4 173557-96-5 173557-97-6 **173557-98-7 173557-99-8**
RL: PRP (Properties)
(rhodostoxin oligosaccharide; structural studies of major hemorrhagin (rhodostoxin) from venom of Calloselasma rhodostoma (Malayan pit viper))
IT 81669-70-7, Metalloproteinase
RL: PRP (Properties)
(structural studies of major hemorrhagin (rhodostoxin) from venom of Calloselasma rhodostoma (Malayan pit viper))

L91 ANSWER 3 OF 7 HCAPLUS COPYRIGHT 2002 ACS
AN 1995:463456 HCAPLUS
DN 122:259079
TI Carbohydrate moieties of porcine 32 kDa enamelin
AU Yamakoshi, Y.
CS Dep. of Biochemistry, Tsurumi Univ., Yokohama, Japan
SO Calcified Tissue International (1995), 56(4), 323-30
CODEN: CTINDZ; ISSN: 0171-967X
PB Springer
DT Journal
LA English
CC 6-3 (General Biochemistry)
AB The structures of asparagine-linked oligosaccharides of porcine 32 kDa enamelin are reported. The oligosaccharides were released by N-oligosaccharide glycopeptidase digestion, and the reducing ends of the oligosaccharides were derivatized with a fluorescent reagent, 2-aminopyridine. The pyridylamino oligosaccharides were sepd. into eight kinds of oligosaccharides. The structures of these oligosaccharides were detd. by a combination of a sequential exoglycosidase digestion and a two-dimensional sugar mapping technique. The oligosaccharides consisted of fucose, galactose, mannose, N-acetylglucosamine, and N-acetylneuraminic acid, and were classified into two groups according to their core-sugar chain structures; one was a biantennary-type and the other was a triantennary-type oligosaccharide. The variation of the oligosaccharides in each of these groups was caused by the differences in the no., the

site, and the mode of linkages of N-acetylneuraminic acid to the core-sugar chains.
ST oligosaccharide structure enamelin
IT Amino acids, biological studies
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(structures of N-linked biantennary- and triantennary-type oligosaccharides of porcine 32 kDa enamelin)
IT Oligosaccharides
RL: PRP (Properties)
(N-linked, structures of N-linked biantennary- and triantennary-type oligosaccharides of porcine 32 kDa enamelin)
IT Proteins, specific or class
RL: PRP (Properties)
(enamelins, structures of N-linked biantennary- and triantennary-type oligosaccharides of porcine 32 kDa enamelin)
IT 78392-81-1 83800-28-6 85541-87-3 108529-39-1 115993-00-5 121283-16-7 162715-12-0 **162715-13-1**
RL: PRP (Properties)
(structures of N-linked biantennary- and triantennary-type oligosaccharides of porcine 32 kDa enamelin)

L91 ANSWER 4 OF 7 HCAPLUS COPYRIGHT 2002 ACS
AN 1992:607864 HCAPLUS
DN 117:207864
TI Carbohydrate structure of a thrombin-like serine protease from Agkistrodon rhodostoma. Structure elucidation of oligosaccharides by methylation analysis, liquid secondary-ion mass spectrometry and proton magnetic resonance
AU Pfeiffer, Guenter; Dabrowski, Ursula; Dabrowski, Janusz; Stirm, Stephan; Strube, Karl Hermann; Geyer, Rudolf
CS Biochem. Inst., Univ. Giessen, Giessen, W-6300, Germany
SO European Journal of Biochemistry (1992), 205(3), 961-78
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
CC 7-5 (Enzymes)
Section cross-reference(s): 33
AB The carbohydrate side chains of the thrombin-like serine protease ancrod from the venom of the Malayan pit viper A. rhodostoma were liberated from tryptic glycopeptides by treatment with peptide-N4-(N-acetyl-.beta.-glucosaminyl)aspargine amidase F and fractionated by HPLC. The glycans obtained were characterized by digestion with exoglycosidases, methylation anal. and, in part, by liq. secondary-ion mass spectrometry and 1H-NMR spectroscopy. The results reveal that this snake venom glycoprotein contains partially truncated di-, tri- and tetraantennary complex-type N-glycans carrying Fuc(.alpha.1-6) residues at the innermost N-acetylglucosamine and solely (.alpha.2-3)-linked sialic acid substituents. As a characteristic feature, ancrod oligosaccharides comprise mainly sialylated Gal.beta.3GlcNAc.beta. lactosamine antennae. Furthermore, a small proportion of the sugar chains were found to carry a NeuAc.alpha.3GalNAc.beta.4GlcNAc.beta. antenna exclusively linked to C-2 of Man(.alpha.1-3) residues of the pentasaccharide core. Thus, many of the glycans found represent novel glycoprotein-N-glycan structures.
ST Agkistrodon thrombinlike serine protease carbohydrate structure; thrombin carbohydrate structure Agkistrodon venom; snake venom thrombin carbohydrate structure
IT Agkistrodon rhodostoma
(thrombin of venom of, carbohydrate structure of)
IT Venoms
(thrombin of, of Agkistrodon rhodostoma, carbohydrate structure of)
IT Oligosaccharides
RL: BIOL (Biological study)

(sialo-, branched, structure of N-linked, of thrombin of Agkistrodon rhodostoma venom)
IT 9002-04-4, Thrombin
RL: BIOL (Biological study)
(carbohydrate structure of serine proteinase related to, of Agkistrodon rhodostoma venom)
IT **144161-29-5 144161-56-8 144161-57-9**
144161-58-0 144161-59-1 144161-60-4
144185-67-1 144185-68-2 144185-69-3
144185-70-6 **144185-73-9 144185-74-0**
144185-75-1 144185-76-2 144249-28-5 144301-31-5
144301-32-6 144302-23-8
RL: BIOL (Biological study)
(of thrombin, of Agkistrodon rhodostoma venom)

L91 ANSWER 5 OF 7 HCAPLUS COPYRIGHT 2002 ACS
AN 1991:490311 HCAPLUS
DN 115:90311
TI Transferrin glycosylation in hypoxia
AU Regoeczi, Erwin; Kay, J. Michael; Chindemi, Paul A.; Zaimi, Ouahida; Suyama, Kaye L.
CS Health Sci. Cent., McMaster Univ., Hamilton, ON, L8N 3Z5, Can.
SO Biochemistry and Cell Biology (1991), 69(4), 239-44
CODEN: BCBIEQ; ISSN: 0829-8211
DT Journal
LA English
CC 14-15 (Mammalian Pathological Biochemistry)
AB The aim of this study was to examine the effect of reduced O2 tension on the glycosylation of transferrin. Rats were placed in a hypobaric chamber (380 mmHg) that corresponded to an altitude of 5486 m above sea level for 21 days. The animals responded with marked increases in hematocrit (from 44 to 76%) and cardiac wt., and with redns. in the concn. of plasma transferrin averaging 15%. Analyses of their plasma transferrin by serial anion-exchange and lectin affinity chromatog. revealed no changes in the extent of glycan branching. However, there was a moderate rise in the proportion of fucosylated transferrin mols. (fucosylation index) and a slight decrease in the transferrin fraction bearing a tetrasialylated biantennary glycan. The fucosylation index correlated pos. with plasma transferrin concns. in the test animals, but not in the controls. In contradistinction to the situation with transferrin, hypoxic rats exhibited a reduced fucosylation index of IgG.
ST hypoxia transferrin fucosylation; glycosylation transferrin hypoxia
IT Hypoxia
(glycosylation of transferrins of blood plasma in)
IT Blood plasma
(glycosylation of transferrins of, in hypoxia)
IT Transferrins
RL: RCT (Reactant); RACT (Reactant or reagent)
(glycosylation of, in blood plasma, in hypoxia)
IT Glycosidation
Oligosaccharides
RL: BIOL (Biological study)
(of transferrins of blood plasma, in hypoxia)
IT Immunoglobulins
RL: RCT (Reactant); RACT (Reactant or reagent)
(G, fucosylation of, in blood plasma, in hypoxia, transferrin glycosylation in relation to)
IT Glycosidation
(fucosidation, of transferrins of blood plasma, in hypoxia)
IT 71496-55-4 73942-65-1 79295-70-8 **135376-92-0** 135376-93-1
135376-94-2
RL: BIOL (Biological study)
(of transferrins of blood plasma, in hypoxia)

L91 ANSWER 6 OF 7 HCAPLUS COPYRIGHT 2002 ACS
AN 1991:159396 HCAPLUS
DN 114:159396
TI Carbohydrate microheterogeneity of rat serotransferrin. Determination of glycan primary structures and characterization of a new type of trisialylated diantennary glycan
AU Spik, Genevieve; Coddeville, Bernadette; Strecker, Gerard; Montreuil, Jean; Regoeczi, Erwin; Chindemi, Paul A.; Rudolph, John R.
CS Lab. Chim. Biol., Univ. Sci. Tech. Lille Flandres-Artois, Villeneuve d'Ascq, F-59655, Fr.
SO European Journal of Biochemistry (1991), 195(2), 397-405
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
CC 6-4 (General Biochemistry)
Section cross-reference(s): 33
AB A previously established procedure was used to isolate from three DEAE-cellulose chromatog. fractions of diferric rat serotransferrin (rTf) subpopulations having discernible affinities for Con A. These entities are designated rTf-1 (not retarded by the Con A column), rTf-2 (retarded), and rTf-3 (bound). Each rTf type was endowed with carbohydrate sufficient to account for a single diantennary glycan/protein mol. Glycan structures were detd. on the glycopeptides by employing GLC/MS and 400-MHz 1H-NMR spectroscopy. All glycans possessed a common, trimannosyl-N,N'-diacetylchitobiose core with or without one L-fucose .alpha.-1,6-linked to the asparagine-linked GlcNAc. However, there were differences in the antennae. Thus, in rTf-3, both antennae were of the disialylated diantennary N-acetyllactosamine type which is frequently encountered in other plasma glycoproteins. However, the .alpha.-1,3-Man-linked antenna in rTf-1 as well as rTf-2 had the sequence: Neu5Ac(.alpha.2-3)Gal(.beta.1-3)[Neu5Ac(.alpha.2-6)]GlcNAc(.beta.1-2)Man. In addn., the .alpha.-1,6-Man-linked antenna deviated in rTf-2 from the std. structure by having the sequence: Neu5Ac(.alpha.2-3)Gal(.beta.1-3)GlcNAc(.beta.1-2)Man. The possible relevance of the above structures to the Con A binding of rTf is discussed. A further prepn., obtained from the most anionic DEAE-cellulose fraction (peak V) of rTf, contained several tetrasialylated diantennary glycans whose precise structures remain to be established in future studies.
ST serotransferrin carbohydrate microheterogeneity; transferrin sero glycan structure
IT Transferrins
RL: PRP (Properties)
(glycan structure of, microheterogeneity in)
IT Oligosaccharides
RL: PRP (Properties)
(sialo-, branched, structure of, of serotransferrin variants)
IT Oligosaccharides
RL: PRP (Properties)
(sialo-, fucose-contg., branched, structure of, of serotransferrin variants)
IT **133157-60-5** 133157-61-6 133179-77-8 **133179-78-9**
133179-79-0 133179-80-3
RL: PRP (Properties)
(structure of, of serotransferrin variants, carbohydrate microheterogeneity in relation to)

L91 ANSWER 7 OF 7 HCAPLUS COPYRIGHT 2002 ACS
AN 1985:467027 HCAPLUS
DN 103:67027
TI The structure of a trisialyl di-antennary N-type glycopeptide obtained from rat plasma hemopexin
AU Bernard, Nicole; Engler, Robert; Strecker, Gerard; Montreuil, Jean; Van

Halbeek, Herman; Vliegenthart, Johannes F. G.
CS Lab. Proteins React. Inflammatoire, UER Biomed. Saint-Peres, Paris, F-75270, Fr.
SO Glycoconjugate Journal (1984), 1(2), 123-40
CODEN: GLJOEW; ISSN: 0282-0080
DT Journal
LA English
CC 6-4 (General Biochemistry)
AB Glycopeptides obtained from rat plasma hemopexin by Pronase digestion were sepd. on Con A-Sepharose into 3 fractions. The lectin-binding fraction was characterized as a mixt. of monosialyl and disialyl diantennary compds. ending in N-acetylneuraminic acid residues .alpha.-(2.fwdarw.6)-linked to galactose in the resp. branches (Bernard, N., et al., 1983). The structures of the glycans in the Con A nonbinding fractions were detd. by a combination of methylation anal. and 500-MHz 1H NMR spectroscopy. Some are triantennary glycans. However, the major component is a trisialyl diantennary glycan. This type of structure was encountered before in some bovine blood coagulation factors as well as in rat .alpha.1-acid glycoprotein, but its 1H NMR parameters are 1st reported here. Furthermore, by methylation anal., the occurrence of the .alpha.-NeuAc-(2.fwdarw.8)-NeuAc (NeuAc = N-acetylneuraminoyl) disaccharide element was demonstrated in a minor part of the carbohydrate moiety of rat hemopexin. This element was also reported previously for rat brain glycopeptides.
ST hemopexin sialooligosaccharide sequence plasma; glycopeptide hemopexin structure
IT Hemopexins
RL: BIOL (Biological study)
(asparagine-linked sialooligosaccharide of, of blood plasma, sequence of)
IT Nuclear magnetic resonance
(of asparagine-linked sialooligosaccharide, of hemopexin)
IT Oligosaccharides
RL: BIOL (Biological study)
(sialo-, asparagine-linked, sequence of, of hemopexin)
IT **97534-26-4**
RL: BIOL (Biological study)
(of hemopexin, of blood plasma)

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L60 ANSWER 1 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 2002:487418 HCAPLUS
DN 137:68127
TI **Erythropoietin** conjugates
IN **Burg, Josef**; Engel, Alfred; Franze, Reinhard; Hilger, Bernd; Schurig, Hartmut Ernst; Tischer, Wilhelm; Wozny, Manfred
PA F. Hoffmann-La Roche Ag, Switz.
SO PCT Int. Appl., 40 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM A61K047-48
CC 63-3 (Pharmaceuticals)
Section cross-reference(s): 2, 16
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 2002049673	A2	20020627	WO 2001-EP14434	20011208

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,

LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH,
PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA,
UG, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH,
CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR,
BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

	AU 2002033230	A5	20020701	AU 2002-33230	20011208
	US 2002115833	A1	20020822	US 2001-14363	20011211
PRAI	EP 2000-127891	A	20001220		
	WO 2001-EP14434	W	20011208		

AB The present invention refers to conjugates of **erythropoietin** with poly(ethylene glycol) comprising an **erythropoietin** glycoprotein having an N-terminal .alpha.-amino group and having the in vivo biol. activity of causing bone marrow cells to increase prodn. of reticulocytes and red blood cells and selected from the group consisting of human **erythropoietin** and analogs thereof which have the sequence of human **erythropoietin** modified by the addn. of from 1 to 6 glycosylation sites or a rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to one poly(ethylene glycol) group of the formula -CO-(CH2)x-(OCH2CH2)m-OR with the -CO of the poly(ethylene glycol) group forming an amide bond with said N-terminal .alpha.-amino group; wherein R is lower alkyl; x is 2 or 3; and m is from about 450 to about 1350.

ST **erythropoietin** conjugate PEG bone marrow proliferation

IT Neoplasm
(anemia from chemotherapy of; glycosylation site-augmented human **erythropoietin** conjugates with PEG)

IT AIDS (disease)
Chemotherapy
(anemia from; glycosylation site-augmented human **erythropoietin** conjugates with PEG)

IT Polyoxyalkylenes, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(**erythropoietin** conjugates; glycosylation site-augmented human **erythropoietin** conjugates with PEG)

IT Kidney, disease
(failure, chronic, anemia from; glycosylation site-augmented human **erythropoietin** conjugates with PEG)

IT Anemia (disease)
Bone marrow
Erythrocyte
Fermentation
Human
Molecular cloning
Protein sequences
Reticulocyte
cDNA sequences
(glycosylation site-augmented human **erythropoietin** conjugates with PEG)

IT Protein motifs
(glycosylation site; glycosylation site-augmented human **erythropoietin** conjugates with PEG)

IT Mutagenesis
(site-directed; glycosylation site-augmented human **erythropoietin** conjugates with PEG)

IT **11096-26-7DP, Erythropoietin**, conjugates
RL: PNU (Preparation, unclassified); THU (Therapeutic use); BIOL (Biological study); PREP (Preparation); USES (Uses)
(glycosylation site-augmented human **erythropoietin** conjugates with PEG)

IT 498-23-7, Citraconic acid **11096-26-7, Erythropoietin**
25322-68-3D, Polyethylene glycol, **erythropoietin** conjugates
RL: RCT (Reactant); RACT (Reactant or reagent)

(glycosylation site-augmented human **erythropoietin** conjugates with PEG)

IT 439058-21-6 439058-22-7 439058-23-8 439058-24-9 439058-25-0 439058-27-2
RL: PRP (Properties)
(unclaimed protein sequence; **erythropoietin** conjugates)

IT 439058-28-3 439058-29-4 439058-30-7 439058-31-8 439058-32-9
RL: PRP (Properties)
(unclaimed sequence; **erythropoietin** conjugates)

L60 ANSWER 2 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:73868 HCAPLUS
DN 134:188284
TI Selective glycopeptide mapping of **erythropoietin** by on-line high-performance liquid chromatography-electrospray ionization mass spectrometry
AU Ohta, M.; Kawasaki, N.; Hyuga, S.; Hyuga, M.; Hayakawa, T.
CS Division of Biological Chemistry and Biologicals, National Institute of Health Sciences, Setagaya-ku, Tokyo, 158-8501, Japan
SO Journal of Chromatography, A (2001), 910(1), 1-11
CODEN: JCRAEY; ISSN: 0021-9673
PB Elsevier Science B.V.
DT Journal
LA English
CC 2-1 (Mammalian Hormones)
AB Selective glycopeptide mapping of recombinant human **erythropoietin** (rhEPO) used as a model glycoprotein was successfully carried out by online HPLC-electrospray ionization mass spectrometry (LC-ESI-MS) using a Vydac C18 column eluted in acetonitrile-1 mM ammonium acetate, pH 6.8. RhEPO expressed in a Chinese hamster ovary clone was exhaustively digested into four glycopeptides and nine peptides with endoproteinase Glu-C. Both glycopeptides and peptides were eluted with trifluoroacetic acid as the eluent, whereas only glycopeptides were eluted selectively with ammonium acetate in the following order: N38, N24, O126, and N83. Furthermore, many glycoforms included in each glycopeptide were found to be sepd. by differences in the nos. of sialic acid and N-**acetyllactosaminyl** repeats. Twenty, 16 and 22 different N-linked oligosaccharides were detd. at Asn 24, 38, and 83, resp., and two different O-linked oligosaccharides were obsd. at Ser 126. The authors' method is simple, rapid, and useful for detg. the carbohydrate structures at each **glycosylation** site and for elucidating the site-specific carbohydrate heterogeneity.
ST **erythropoietin** glycopeptide oligosaccharide mapping **glycosylation** site HPLC MS method
IT Mass spectrometry
(HPLC combined with, electrospray ionization MS; glycopeptide mapping of **erythropoietin** by online high-performance liq. chromatog.-electrospray ionization mass spectrometry)
IT Oligosaccharides, biological studies
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(N-linked; glycopeptide mapping of **erythropoietin** by online high-performance liq. chromatog.-electrospray ionization mass spectrometry)
IT Oligosaccharides, biological studies
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(O-linked; glycopeptide mapping of **erythropoietin** by online high-performance liq. chromatog.-electrospray ionization mass spectrometry)
IT Glycoproteins, specific or class
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP

(Properties); BIOL (Biological study); PROC (Process)
(glycopeptide mapping of **erythropoietin** by online high-performance liq. chromatog.-electrospray ionization mass spectrometry)

IT Protein motifs
(**glycosylation** site; glycopeptide mapping of **erythropoietin** by online high-performance liq. chromatog.-electrospray ionization mass spectrometry)

IT HPLC
(mass spectrometry combined with, electrospray ionization MS; glycopeptide mapping of **erythropoietin** by online high-performance liq. chromatog.-electrospray ionization mass spectrometry)

IT 75-05-8, Acetonitrile, uses 76-05-1, Trifluoroacetic acid, uses 131-48-6, N-Acetyl neuraminic acid 631-61-8, Ammonium acetate 137010-42-5
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(glycopeptide mapping of **erythropoietin** by online high-performance liq. chromatog.-electrospray ionization mass spectrometry)

IT **32181-59-2, N-Acetyllactosamine**
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)
(glycopeptide mapping of **erythropoietin** by online high-performance liq. chromatog.-electrospray ionization mass spectrometry)

IT **11096-26-7, Erythropoietin**
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(glycopeptide mapping of **erythropoietin** by online high-performance liq. chromatog.-electrospray ionization mass spectrometry)

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L60 ANSWER 3 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 2001:31360 HCAPLUS
DN 134:105827
TI **Erythropoietin** derivatives

IN **Burg, Josef**; Hilger, Bernd; Josel, Hans-Peter
PA F. Hoffmann-La Roche A.-G., Switz.
SO PCT Int. Appl., 40 pp.
CODEN: PIXXD2
DT Patent
LA English
IC ICM A61K047-48
CC 63-3 (Pharmaceuticals)
Section cross-reference(s): 2, 34
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2001002017	A2	20010111	WO 2000-EP6009	20000628
	W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
	US 6340742	B1	20020122	US 2000-604871	20000628
	EP 1196443	A2	20020417	EP 2000-951312	20000628
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
	BR 2000012138	A	20020507	BR 2000-12138	20000628
	NO 2001006304	A	20020219	NO 2001-6304	20011221
PRAI	US 1999-142243P	P	19990702		
	US 1999-147452P	P	19990805		
	US 1999-151454P	P	19990830		
	WO 2000-EP6009	W	20000628		

AB **Erythropoietin** glycoprotein conjugates are disclosed, said conjugates comprise an **erythropoietin** glycoprotein having at least one free amino group and having the in vivo biol. activity of causing bone marrow cells to increase prodn. of reticulocytes and red blood cells and selected from the group consisting of human **erythropoietin** and analogs thereof which have the primary structure of human **erythropoietin** modified by the addn. of from 1 to 6 glycosylation sites or by the rearrangement of at least one glycosylation site; said glycoprotein being covalently linked to form one to three lower-alkoxy poly(ethylene glycol) groups, each poly(ethylene glycol) group being covalently linked to the glycoprotein via a linker of the formula -C(O)-X-S-Y- with the C(O) of the linker forming an amide bond with one of said amino groups, wherein X and Y are as defined in the description and claims, the av. mol. wt. of each poly(ethylene glycol) moiety is from about 20 kilodaltons to about 40 kilodaltons, and the mol. wt. of the conjugate is from about 51 kilodaltons to about 175 kilodaltons.

ST **erythropoietin** polyethylene glycol conjugate hematopoiesis stimulation

IT Chemotherapy
(anemia from; **erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT Polyoxyalkylenes, biological studies
RL: PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**erythropoietin** conjugates; **erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT AIDS (disease)
Anemia (disease)
Coupling agents
Erythrocyte

Erythropoiesis
Protein sequences
Reticulocyte
(**erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT Glycoproteins, general, biological studies
RL: PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT Gene
(expression, **erythropoietin**-induced; **erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT Kidney, disease
(failure, chronic; **erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT Protein motifs
(glycosylation site; **erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT Bone marrow
(hematopoiesis in; **erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT **96024-34-9**, **Erythropoietin** (human clone .lambda.HEPOFL13 protein moiety reduced) **134547-95-8**, 1-165-**Erythropoietin** (human clone .lambda.HEPOFL13 protein moiety reduced)
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)
(amino acid sequence; **erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT 9002-61-3, Human chorionic gonadotropin
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(carboxy terminal sequence of; **erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT 66090-83-3
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PEP (Physical, engineering or chemical process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)
(**erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

IT **11096-26-7D**, **Erythropoietin**, conjugates 25322-68-3D, **erythropoietin** conjugates
RL: PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(**erythropoietin** derivs. for increasing prodn. of erythrocytes and reticulocytes)

L60 ANSWER 4 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 2000:661050 HCAPLUS
DN 134:39085
TI Application of Liquid Chromatography/Mass Spectrometry and Liquid Chromatography with Tandem Mass Spectrometry to the Analysis of the Site-Specific Carbohydrate Heterogeneity in Erythropoietin
AU Kawasaki, Nana; Ohta, Miyako; Hyuga, Sumiko; Hyuga, Masashi; Hayakawa, Takao
CS Division of Biological Chemistry and Biologicals, National Institute of Health Sciences, Kamiyoga, Setagaya-ku, Tokyo, 158-8501, Japan
SO Analytical Biochemistry (2000), 285(1), 82-91
CODEN: ANBCA2; ISSN: 0003-2697

PB Academic Press
DT Journal
LA English
CC 9-16 (Biochemical Methods)
Section cross-reference(s): 6
AB High-performance liq. chromatog. with electrospray ionization mass spectrometry (LC/MS) and liq. chromatog. with tandem mass spectrometry (LC/MS/MS) were applied to the anal. of the site-specific carbohydrate heterogeneity in **erythropoietin** (**EPO**) used as a model of the sialylated glycoprotein. N-linked oligosaccharides were released from recombinant human **EPO** expressed in Chinese hamster ovary cells enzymically and reduced with NaBH4. Many different sialylated oligosaccharides of **EPO** were sepd. and characterized by LC/MS equipped with a graphitized carbon column (GCC). **Glycosylation** sites and the preliminary **glycosylation** pattern at each **glycosylation** site were detd. by LC/MS of endoproteinase Glu-C-digested **EPO**. The detailed site-specific carbohydrate heterogeneity caused by the differences in the mol. wt., branch, linkage, and sequence was elucidated by GCC-LC/MS of the N-linked oligosaccharides released from the isolated glycopeptides. Structural details of the isomers were analyzed by LC/MS/MS, and it was indicated that di- and tri-sialylated tetra-antennary oligosaccharides are attached to Asn24, 38, and 83, whereas their isomers, di- and tri-sialylated tri-antennary oligosaccharides contg. N-**acetyllactosamines**, are combined with Asn24. Our method is useful for the detn. of **glycosylation** sites, the site-specific carbohydrate heterogeneity of glycoproteins, and the carbohydrate structure. (c) 2000 Academic Press.
ST liq chromatog mass spectrometry glycoprotein erythropoietin carbohydrate heterogeneity
IT Animal cell line
(CHO, expressing recombinant human erythropoietin; application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in erythropoietin)
IT Oligosaccharides, properties
RL: PEP (Physical, engineering or chemical process); PRP (Properties); PROC (Process)
(N-linked; application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in erythropoietin)
IT Heterogeneity
Protein sequences
Simulation and Modeling, biological
(application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in erythropoietin)
IT Carbohydrates, properties
Sialooligosaccharides
RL: PEP (Physical, engineering or chemical process); PRP (Properties); PROC (Process)
(application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in erythropoietin)
IT Protein motifs
(**glycosylation** site; application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in **erythropoietin**)
IT Mass spectrometry
Tandem mass spectrometry
(liq. chromatog. combined with; application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in erythropoietin)
IT Liquid chromatography
(mass spectrometry combined with; application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in erythropoietin)
IT Glycoproteins, general, properties

RL: PRP (Properties)
(sialylated; application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in erythropoietin)
IT 11096-26-7, Erythropoietin
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)
(application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in erythropoietin)
IT 137010-42-5
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(application of LC/MS and LC with tandem mass spectrometry to anal. of site-specific carbohydrate heterogeneity in erythropoietin)
RE.CNT 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L60 ANSWER 5 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 2000:601426 HCAPLUS
DN 133:247384
TI Asn-linked sugar chain structures of recombinant human thrombopoietin produced in Chinese hamster ovary cells
AU Inoue, Noboru; Watanabe, Toshinori; Kutsukake, Toshiko; Saitoh, Hiroyuki; Tsumura, Haruhiko; Arai, Hirofumi; Takeuchi, Makoto
CS Pharmaceutical Development Laboratory, KIRIN Brewery Co., Ltd., Gunma, 370-0013, Japan
SO Glycoconjugate Journal (2000), Volume Date 1999, 16(11), 707-718
CODEN: GLJOEW; ISSN: 0282-0080
PB Kluwer Academic Publishers
DT Journal
LA English
CC 2-2 (Mammalian Hormones)
AB Human thrombopoietin (TPO) that regulates the nos. of megakaryocytes and platelets is a heavily N- and O- **glycosylated** glycoprotein hormone with partial homol. to human **erythropoietin** (**EPO**). The authors prepd. recombinant human TPO produced in Chinese hamster ovary (CHO) cells and analyzed the sugar chain structures quant. using

2-aminobenzamide labeling, sequential glycosidase digestion and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS). The authors found bi-, tri- and tetraantennary complex-type sugar chains with one or two N-**acetyllactosamine** repeats, which are common to recombinant human **EPO** produced in CHO cells. There were triantennary sugar chains with one or two N-**acetyllactosamine** repeats that were specific to the recombinant human TPO, and their distributions of branch structures were also different. These results suggested that proximal protein structure should det. the branch structure of Asn-linked sugar chains in addn. to the glycosyltransferases subset.

ST recombinant thrombopoietin sugar chain structure

IT Molecular structure, natural product
(Asn-linked sugar chain structures of recombinant human thrombopoietin produced in Chinese hamster ovary cells)

IT Oligosaccharides, biological studies
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(Asn-linked sugar chain structures of recombinant human thrombopoietin produced in Chinese hamster ovary cells)

IT 78392-81-1 83412-55-9 84813-89-8 107688-07-3 115142-59-1 115142-61-5 126946-87-0 176449-49-3 176449-50-6 294856-68-1
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(Asn-linked sugar chain structures of recombinant human thrombopoietin produced in Chinese hamster ovary cells)

IT 9014-42-0, Thrombopoietin
RL: PRP (Properties)
(Asn-linked sugar chain structures of recombinant human thrombopoietin produced in Chinese hamster ovary cells)

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L60 ANSWER 6 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1999:308787 HCAPLUS
DN 131:161506
TI Biodegradable recombinant human **erythropoietin** loaded microspheres prepared from linear and star-branched block copolymers: Influence of encapsulation technique and polymer composition on particle characteristics
AU Pistel, K. F.; Bittner, B.; **Koll, H.**; Winter, G.; Kissel, T.
CS Department of Pharmaceutics and Biopharmacy, Philipps-University, Marburg, Germany
SO Journal of Controlled Release (1999), 59(3), 309-325
CODEN: JCREEC; ISSN: 0168-3659
PB Elsevier Science Ireland Ltd.
DT Journal
LA English
CC 63-5 (Pharmaceuticals)
AB Recombinant human **erythropoietin** (**EPO**) and fluorescein isothiocyanate labeled dextran (FITC-dextran) loaded microspheres were prepd. by a modified W/O/W double-emulsion technique. Biodegradable linear ABA block copolymers consisting of poly(L-lactide-co-glycolide) A blocks attached to central poly(ethyleneoxide) (PEO) B blocks and star-branched AB block copolymers contg. A blocks of poly(L-lactide) or poly(L-lactide-co-glycolide) and star-branched poly(ethyleneoxide) B blocks were investigated for their potential as sustained release drug delivery systems. Microsphere characteristics were strongly influenced by the polymer compn. In the case of the linear block copolymers, a reduced lactic acid content in a linear block copolymer yielded smaller particles, a lower encapsulation efficiency, and a higher initial drug release both in the case of **EPO** and FITC-dextran. The investigation of the effects of several manufg. parameters on microsphere formation showed that the process temp. plays an important role. Microsphere formation in a +1.degree.C environment resulted in higher drug loadings without increasing the amt. of residual dichloromethane inside the particles. Other parameters such as the homogenization of the primary W/O emulsion and of the W/O/W double-emulsion have less impact on microsphere characteristics. Branched block copolymers contg. star-shaped PEO also showed potential for the prepn. of drug loaded microspheres. A certain amt. of glycolic acid in the copolymer was necessary for the successful prepn. of non-aggregating microspheres at room temp. Again, the processing temp. strongly affected particle characteristics. Microsphere prepn. at +1.degree.C allows the formation of microspheres from a polymer not contg. glycolic acid, a result which could not be achieved at room temp. Moreover, compared to microsphere formation at room temp., the effective FITC-dextran loading was increased. Concerning the **EPO** loaded microspheres, the amt. of **EPO** aggregated was comparable to that using the linear ABA polymers. A continuous release of the protein from these star-shaped polymers could not be achieved. In conclusion, apart from microsphere prepn. in a +1.degree.C environment the choice of the polymer represents the main factor for a successful entrapment of proteins into biodegradable microspheres.
ST biodegradable polyester polyoxyalkylene copolymer microsphere **erythropoietin**
IT Polymers, biological studies
RL: PEP (Physical, engineering or chemical process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(biodegradable; encapsulation technique and polymer compn. effect on particle characteristics of biodegradable recombinant human

erythropoietin loaded microspheres prepd. from linear and star-branched block copolymers)

IT Encapsulation
Particle size
(encapsulation technique and polymer compn. effect on particle characteristics of biodegradable recombinant human **erythropoietin** loaded microspheres prepd. from linear and star-branched block copolymers)

IT Drug delivery systems
(microspheres; encapsulation technique and polymer compn. effect on particle characteristics of biodegradable recombinant human **erythropoietin** loaded microspheres prepd. from linear and star-branched block copolymers)

IT Polyoxyalkylenes, biological studies
Polyoxyalkylenes, biological studies
RL: PEP (Physical, engineering or chemical process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(polyester-; encapsulation technique and polymer compn. effect on particle characteristics of biodegradable recombinant human **erythropoietin** loaded microspheres prepd. from linear and star-branched block copolymers)

IT Polyesters, biological studies
Polyesters, biological studies
RL: PEP (Physical, engineering or chemical process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(polyoxyalkylene-; encapsulation technique and polymer compn. effect on particle characteristics of biodegradable recombinant human **erythropoietin** loaded microspheres prepd. from linear and star-branched block copolymers)

IT **11096-26-7, Erythropoietin** 60842-46-8, Fitc-dextran 113497-67-9 131151-09-2
RL: PEP (Physical, engineering or chemical process); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(encapsulation technique and polymer compn. effect on particle characteristics of biodegradable recombinant human **erythropoietin** loaded microspheres prepd. from linear and star-branched block copolymers)

RE.CNT 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD

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AN 1999:96350 HCAPLUS
DN 130:149562
TI Production of **erythropoietin** by endogenous gene activation of human cells
IN Stern, Anne; Brandt, Michael; Honold, Konrad; Auer, Johannes; **Koll, Hans**
PA Boehringer Mannheim G.m.b.H., Germany
SO PCT Int. Appl., 70 pp.
CODEN: PIXXD2
DT Patent
LA German
IC ICM C12N015-12
ICS C12N015-85; C12N015-62; C12N015-90; C12N005-10; C07K014-505; A61K038-18
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 16
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI	WO 9905268	A1	19990204	WO 1998-EP4590	19980722	<--
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM					
	RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG					
	DE 19753681	A1	19990722	DE 1997-19753681	19971203	<--
	AU 9889786	A1	19990216	AU 1998-89786	19980722	<--
	ZA 9806515	A	20000124	ZA 1998-6515	19980722	
	ZA 9806516	A	20000124	ZA 1998-6516	19980722	
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	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI					
	BR 9811031	A	20000808	BR 1998-11031	19980722	<--

PI JP 2001511343 T2 20010814 JP 2000-504243 19980722 <--
WO 9928455 A1 19990610 WO 1998-EP7819 19981202 <--
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
AU 9920518 A1 19990616 AU 1999-20518 19981202 <--
ZA 9811003 A 20000602 ZA 1998-11003 19981202 <--
ZA 9811004 A 20000602 ZA 1998-11004 19981202 <--
EP 1036179 A1 20000920 EP 1998-965223 19981202 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI
JP 2001525342 T2 20011211 JP 2000-523332 19981202 <--
CA 2309810 AA 19990610 CA 1998-2309810 19981203 <--
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W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
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R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI
BR 9813391 A 20001010 BR 1998-13391 19981203 <--
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WO 1998-EP7876 W 19981203 <--
US 2000-463380 A1 20000121

AB The invention concerns human cells which, owing to the activation of the endogenous human **erythropoietin** gene, can produce **erythropoietin** (**EPO**) in sufficient quantities and degree of purity to allow human **EPO** to be economically produced as a pharmaceutical prepn. The invention also concerns a process for producing such human **EPO**-producing cells, DNA-constructs for activating the endogenous **EPO** gene in human cells and a process for the large-scale prodn. of **EPO** in human cells. A HeLa S3 cell contg. **erythropoietin** genes fused to a cytomegalovirus immediate early promoter and enhancer was produced by homologous recombination. Optimization of the **erythropoietin** gene expression involved alteration of the signal sequence, shortening of the distance between the cytomegalovirus promoter and translation start site, and amplification of the gene. A recombinant cell line producing >7000 ng **erythropoietin**/mL/106 cells/24 h was obtained. The **erythropoietin** was purified by a series of chromatog. steps (affinity, hydrophobic interaction, hydroxyapatite, reverse phase HPLC) to produce **erythropoietin** with specific activity >100,000 units/mg.

ST **erythropoietin** manuf recombinant human cell cytomegalovirus immediate early promoter

IT Animal cell line
(HT-1080; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT Animal cell line
(Namalwa; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT HeLa cell
(S3; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT Gene, animal
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(for **erythropoietin**, activation of; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT Recombination, genetic
(homologous; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT Animal cell
(human; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT Promoter (genetic element)
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(immediate early, of cytomegalovirus, for activation of **erythropoietin** gene; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT Plasmid vectors
(p189; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT Fermentation
(prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT Genetic element
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(signal sequence, modified; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT Promoter (genetic element)
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(viral, for activation of **erythropoietin** gene; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT 75432-66-5, Blue Sepharose
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(Blue Sepharose; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT 9002-03-3P, Dihydrofolate reductase
RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)
(gene for, as amplification gene; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT 62213-36-9P, Neomycin phosphotransferase
RL: BPN (Biosynthetic preparation); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation); PROC (Process)
(gene for, as selectable marker; prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT **11096-26-7P, Erythropoietin**

RL: BPN (Biosynthetic preparation); BIOL (Biological study); PREP (Preparation)
(prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT 72980-05-3
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(prodn. of **erythropoietin** by endogenous gene activation of human cells)
IT 220271-95-4 220271-96-5 220271-97-6 220271-98-7
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(signal peptide N-terminus; prodn. of **erythropoietin** by endogenous gene activation of human cells)
RE.CNT 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD
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L60 ANSWER 8 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1998:791475 HCAPLUS
DN 130:49404
TI Peptide capillary zone electrophoresis mass spectrometry of recombinant human erythropoietin. An evaluation of the analytical method
AU Boss, Hollis J.; Watson, Daniel B.; Rush, Robert S.
CS Department Protein Structure, M/S 14-2-E, AMGEN Inc., Thousand Oaks, CA, 91320, USA
SO Electrophoresis (1998), 19(15), 2654-2664
CODEN: ELCTDN; ISSN: 0173-0835
PB Wiley-VCH Verlag GmbH
DT Journal
LA English
CC 9-7 (Biochemical Methods)
AB An evaluation of capillary zone electrophoresis-mass spectrometry as an anal. methodol. for the sepn. and characterization of complex glycopeptides and nonglycopeptide structures was performed. The evaluation employed endoproteinase V8 digested recombinant human **erythropoietin** (rHuEPO) that was further fractionated by reverse phase chromatog. The peptides were subjected to sequence anal. and evaluated by capillary electrophoresis, with or without mass detection, for peptide purity. The peptide mass detd. from the sequence was then compared to the mass obtained from CZE-MS. **Glycosylation** sites and carbohydrate branch patterns were easily detd., site specific microheterogeneity (either O-**acetylation** of N-**acetylneuraminic** acids or **lactosamine** extensions of the carbohydrate chain length) was assessed directly, **glycosylation** site occupancy was evaluated qual., and nonglycopeptides were resolved and analyzed online with ease. Incomplete peptide digestion products were detected and identified by CZE-MS. Protein sequence coverage by CZE-MS was 98.2% complete from a single map. Off-line evaluation of peptide

purity by CZE greatly aided the interpretation of multiple sequence anal. and, in validating that, the CZE-MS was detecting all peptides present. All off-line CZE and online CZE-MS expts. employed a capillary that was dynamically coated with polybrene in the presence of polyethylene glycol; sepns. were conducted in 0.67 M formic acid.

ST capillary zone electrophoresis mass spectrometry erythropoietin

IT Capillary zone electrophoresis
Mass spectrometry
(evaluation of peptide capillary zone electrophoresis mass spectrometry of recombinant human erythropoietin)

IT Glycoproteins, general, analysis
RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)
(evaluation of peptide capillary zone electrophoresis mass spectrometry of recombinant human erythropoietin)

IT 11096-26-7, Erythropoietin
RL: ANT (Analyte); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study)
(evaluation of peptide capillary zone electrophoresis mass spectrometry of recombinant human erythropoietin)

RE.CNT 65 THERE ARE 65 CITED REFERENCES AVAILABLE FOR THIS RECORD

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AN 1998:705278 HCAPLUS
DN 130:144059
TI **Erythropoietin** loaded microspheres prepared from biodegradable LPLG-PEO-LPLG triblock copolymers: protein stabilization and in-vitro release properties
AU Morlock, Michael; Kissel, Thomas; Li, You Xin; **Koll, Hans**; Winter, Gerhard
CS Department of Pharmaceutics and Biopharmacy, Philipps University, Marburg, D-35032, Germany
SO Journal of Controlled Release (1998), 56(1-3), 105-115
CODEN: JCREEC; ISSN: 0168-3659
PB Elsevier Science B.V.
DT Journal
LA English
CC 63-5 (Pharmaceuticals)
AB Biodegradable microspheres contg. recombinant human **erythropoietin** (**EPO**) were prepd. from ABA triblock copolymers, consisting of hydrophobic poly(L-lactic-co-glycolic acid) A blocks and hydrophilic polyethylene oxide (PEO) B blocks. Different polymer compns. were studied for the microencapsulation of **EPO** using a modified double-emulsion process (W/O/W). The encapsulation efficiency for **EPO**, ranging from 72% to 99% was quite acceptable. The formation of high mol. wt. **EPO** aggregates, however, was higher than in poly(DL-lactide-co-glycolide) (PLG) microparticles. Using different excipients with known protein stabilizing properties, such as Bovine Serum Albumin (BSA), Poly-L-histidine (PH), Poly-L-arginine (PA) or a combination of PA with Dextran 40 (D40), the **EPO** aggregate content was significantly reduced to <5% of the encapsulated **EPO** . In contrast to PLG, ABA triblock copolymers contg. >7 mol % PEO, allowed a continuous release of **EPO** from microspheres for up to 2 wk under in-vitro conditions. The release profile was comparable to FITC-Dextran 40 kDa (FD 40) loaded microspheres in the initial release phase, while **EPO** release was leveling off at later time points. BSA addnl. prolonged the **EPO** release, while blends of PLG and PEO did not generate continuous **EPO** release profiles. LPLG-PEO-LPLG triblock-copolymers (35 mol % PEO; 30 kDa) in combination

with 5% BSA yielded both an acceptable level of **EPO** aggregates and a continuous release profile under in-vitro conditions for up to 2 wk. The formation of **EPO** aggregates at later time points is probably induced by acidic cleavage products of the biodegradable polymer and requires further optimization of the ABA polymer compn.

ST **erythropoietin** microsphere PEG lactate glycolate copolymer; biodegradable PEG polyester microsphere **erythropoietin**

IT Polymers, biological studies
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(biodegradable; protein stabilization and in-vitro release properties of **erythropoietin** loaded microspheres prepd. from biodegradable LPLG-PEO-LPLG triblock copolymers)

IT Drug delivery systems
(microspheres; protein stabilization and in-vitro release properties of **erythropoietin** loaded microspheres prepd. from biodegradable LPLG-PEO-LPLG triblock copolymers)

IT Polyoxyalkylenes, biological studies
Polyoxyalkylenes, biological studies
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(polyester-; protein stabilization and in-vitro release properties of **erythropoietin** loaded microspheres prepd. from biodegradable LPLG-PEO-LPLG triblock copolymers)

IT Polyesters, biological studies
Polyesters, biological studies
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(polyoxyalkylene-; protein stabilization and in-vitro release properties of **erythropoietin** loaded microspheres prepd. from biodegradable LPLG-PEO-LPLG triblock copolymers)

IT Dissolution rate
(protein stabilization and in-vitro release properties of **erythropoietin** loaded microspheres prepd. from biodegradable LPLG-PEO-LPLG triblock copolymers)

IT Proteins, general, biological studies
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(protein stabilization and in-vitro release properties of **erythropoietin** loaded microspheres prepd. from biodegradable LPLG-PEO-LPLG triblock copolymers)

IT Albumins, biological studies
RL: MOA (Modifier or additive use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(serum; protein stabilization and in-vitro release properties of **erythropoietin** loaded microspheres prepd. from biodegradable LPLG-PEO-LPLG triblock copolymers)

IT 24937-47-1, Poly(L-arginine) 25212-18-4, Poly(L-arginine) 26062-48-6, Poly(L-histidine) 26854-81-9
RL: MOA (Modifier or additive use); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(protein stabilization and in-vitro release properties of **erythropoietin** loaded microspheres prepd. from biodegradable LPLG-PEO-LPLG triblock copolymers)

IT **11096-26-7, Erythropoietin** 160036-33-9, Propanoic acid, 2-hydroxy-, (2S)-, polymer with .alpha.-hydro-.omega.-hydroxypoly(oxy-1,2-ethanediyl) and hydroxyacetic acid, block
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(protein stabilization and in-vitro release properties of **erythropoietin** loaded microspheres prepd. from biodegradable LPLG-PEO-LPLG triblock copolymers)

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L60 ANSWER 10 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1998:442374 HCAPLUS
DN 129:72094
TI Recombinant human **erythropoietin** (rhEPO) loaded poly(lactide-co-glycolide) microspheres. Influence of the encapsulation technique and polymer purity on microsphere characteristics
AU Bittner, Beate; Morlock, Michael; **Koll, Hans**; Winter, Gerhard; Kissel, Thomas
CS Dep. Pharmaceutics Biopharmacy, Philipps-Univ., Marburg, D-35032, Germany
SO European Journal of Pharmaceutics and Biopharmaceutics (1998), 45(3), 295-305
CODEN: EJPBEL; ISSN: 0939-6411
PB Elsevier Science Ireland Ltd.
DT Journal
LA English
CC 63-5 (Pharmaceuticals)
AB Recombinant human **erythropoietin** (**EPO**) and fluorescein isothiocyanate-labeled dextran (FITC-dextran) loaded biodegradable microspheres were prepd. from poly(lactide-co-glycolide) (PLG) by a modified spray-drying technique. This microencapsulation method was compared with the water-in-oil-in-water (w/o/w) double-emulsion method. As expected, microsphere morphol., particle size, and particle size distribution strongly depended on the prodn. process. The spray-drying method was found to have a no. of advantages compared to the w/o/w double-emulsion technique. The content of residual dichloromethane (DCM) in the final product was lower in case of the microspheres prepd. by spray-drying. Concerning **EPO** loaded microspheres, spray-drying yielded higher encapsulation efficiencies. Although the microspheres obtained by spray-drying are subjected to intensive mech. and thermal stress during the prepn., the amt. of aggregates of **EPO** in PLG microspheres were not increased compared to the w/o/w technique. Depending on the manufg. method, addn. of cyclic DL-lactide dimers (referred to as monomers in the following) affected the in vitro release profiles of **EPO** and FITC-dextran from PLG microspheres. Using differential scanning calorimetry it was shown that these low mol. wt. substances only were present inside the microspheres produced by spray-drying. DL-Lactide reduced the initial burst release of both **EPO** and FITC-dextran. While the following release period of **EPO** was not affected by the DL-lactide content, a more linear FITC-dextran release pattern could be achieved. It can be concluded that

the spray-drying technique provides a no. of advantages compared to the w/o/w method. The modulation of protein release using low mol. wt. additives is of particular interest for parenteral depot systems.

ST **erythropoietin** delivery microencapsulation technique polymer

IT Encapsulation
(microencapsulation; influence of encapsulation technique and polymer purity on microsphere characteristics)

IT Drug delivery systems
(microspheres; influence of encapsulation technique and polymer purity on microsphere characteristics)

IT Drying
(spray; properties of microspheres prepd. by a spray-drying technique)

IT Emulsions
Emulsions
(water-in-oil-in-water; properties of microspheres prepd. by w/o/w double emulsion technique)

IT 60842-46-8, FITC-dextran
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**erythropoietin** and dextran loaded poly(lactide-co-glycolide) microspheres)

IT **11096-26-7, Erythropoietin**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(**erythropoietin** loaded poly(lactide-co-glycolide) microspheres)

IT 26780-50-7, Poly(lactide-co-glycolide)
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(influence of encapsulation technique and polymer purity on microsphere characteristics)

IT 75-09-2, Dichloromethane, biological studies
RL: BUU (Biological use, unclassified); OCU (Occurrence, unclassified); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(residual DCM in microspheres in dependence of manufg. method)

L60 ANSWER 11 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1998:114751 HCAPLUS
DN 128:226663
TI Effect of ammonium ion on glycoform of **erythropoietin** produced by recombinant CHO cells using lectin-blotting technique
AU Chang, Kern Hee; Kim, Kyung Soo; Kim, Jung Hoe
CS Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Taejon, S. Korea
SO Animal Cell Technology: Basic & Applied Aspects, Proceedings of the Annual Meeting of the Japanese Association for Animal Cell Technology, 9th, Yokohama, Sept. 1-4, 1996 (1998), Meeting Date 1996, 361-365. Editor(s): Nagai, Kazuo; Wachi, Masaaki. Publisher: Kluwer, Dordrecht, Neth. CODEN: 65RGAA
DT Conference
LA English
CC 2-10 (Mammalian Hormones)
AB The **glycosylation** pattern of **erythropoietin** (**EPO**), produced by recombinant CHO cells, was studied using rapid and simple method of "Lectin-blotting". The authors used three different lectins in this expt.; MAA (Maackia amurensis agglutinin), RCA (Ricinus communis agglutinin), DSA (Datura stramonium agglutinin) which binds to terminal sialic acid, galactose and N-**acetyllactosamine** chain, resp. The effect of ammonium ion on **glycosylation** of **EPO** was examd. since it accumulates in the medium as a byproduct mainly resulted from the glutamine metab. Consequently, treatment of ammonium chloride from 8 mM to above substantially inhibited the sialylation of terminal galactose residue.

ST ammonium ion **erythropoietin** galactose sialylation
IT **Sialylation**
(ammonium ion inhibition of sialylation of **erythropoietin** terminal galactose residue)
IT 12125-02-9, Ammonium chloride, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(ammonium ion inhibition of sialylation of **erythropoietin** terminal galactose residue)
IT 59-23-4, D-Galactose, biological studies **11096-26-7**, **Erythropoietin**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(ammonium ion inhibition of sialylation of **erythropoietin** terminal galactose residue)

L60 ANSWER 12 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1997:801500 HCAPLUS
DN 128:151077
TI Identification of essential histidine residues in UDP-N-**acetyl**-D-**galactosamine**:polypeptide N-**acetylgalactosaminyltransferase**-T1
AU Wragg, Stephanie; Hagen, Fred K.; Tabak, Lawrence A.
CS Departments of Dental Research and Biochemistry and Biophysics, School of Medicine and Dentistry, University of Rochester, Rochester, NY, 14642, USA
SO Biochemical Journal (1997), 328(1), 193-197
CODEN: BIJOAK; ISSN: 0264-6021
PB Portland Press Ltd.
DT Journal
LA English
CC 7-5 (Enzymes)
AB Polypeptide N-**acetylgalactosaminyltransferase** (I) catalyzes the initial step of mucin-type O-**glycosylation**. The activity of bovine I isoenzyme T1 (I-T1) was inhibited by modification with di-Et pyrocarbonate (DEPC). I-T1 activity was partially restored by hydroxylamine treatment, indicating that one of the reactive residues was His. I-T1 was protected against DEPC inactivation when UDP-GalNAc and **EPO**-G, a peptide pseudosubstrate PPDAAGAAPLR, were simultaneously present, whereas the presence of **EPO**-G alone did not alter DEPC inactivation. However, inclusion of UDP-GalNAc alone potentiated DEPC-inhibition of the enzyme, suggesting that UDP-GalNAc binding changes the accessibility or reactivity of an essential His residue. Deletion of the 1st 56 amino acids (including 1 His residue) yielded a fully active secreted form of bovine I-T1. Each of the 14 remaining His residues in I-T1 were mutated to Ala residues, and the recombinant mutants were recovered from COS7 cells. Mutants H211A and H344A resulted in recombinant proteins with no detectable enzymic activity. A significant decrease in the initial rate of GalNAc transfer to the substrate was obsd. with mutants H125A and H341A (1 and 6% of wild-type activity, resp.). Mutation of the remaining 10 His residues yielded mutants that were indistinguishable from the wild-type enzyme. Mutagenesis and SDS-PAGE anal. of all N-**glycosylation** sequons revealed that residues Asn-95 and Asn-552 were occupied by N-linked sugars in COS7 cells. Ablation of either site did not perturb enzyme biosynthesis or enzyme activity.
ST polypeptide **acetylgalactosaminyltransferase** essential histidine modification
IT Protein motifs
(glycosylation site; Asn-95 and Asn-552 as N-glycosylation sites in polypeptide N-**acetylgalactosaminyltransferase**-T1)
IT Michaelis constant
(of polypeptide N-**acetylgalactosaminyltransferase**-T1 wild-type and mutant forms)

IT 71-00-1, L-Histidine, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(essential; identification and chem. modification of essential His residues in polypeptide N-**acetylgalactosaminyltransferase**-T1)
IT 9075-15-4, Polypeptide N-**acetylgalactosaminyltransferase**
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(isoenzyme T1; identification and chem. modification of essential His residues in polypeptide N-**acetylgalactosaminyltransferase**-T1)

L60 ANSWER 13 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1997:601111 HCAPLUS
DN 127:288284
TI Glycopeptide profiling of human urinary **Erythropoietin** by matrix-assisted laser desorption/ionization mass spectrometry
AU Rahbek-Nielsen, Henrik; Roepstorff, Peter; Reischl, Heinz; Wozny, Manfred; **Koll, Hans; Haselbeck, Anton**
CS Department of Molecular Biology, Odense University, Odense, DK-5230, Den.
SO Journal of Mass Spectrometry (1997), 32(9), 948-958
CODEN: JMSPFJ; ISSN: 1076-5174
PB Wiley
DT Journal
LA English
CC 2-2 (Mammalian Hormones)
AB The site-specific glycan heterogeneity of human urinary **erythropoietin** was investigated by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS). Owing to the small amt. of protein available, a strategy combining optimal sensitivity and specificity was used. **Erythropoietin** was reduced, S-alkylated and digested with endoproteinase Lys C. The peptides were sepd. by reversed-phase high-performance liq. chromatog. and the mol. masses of the peptides detd. by MALDI-MS. The peptides were identified by comparing the exptl. masses with the masses predicted from the cDNA derived amino acid sequence. Glycopeptides were identified from the mass spectra based on the peak pattern caused by the glycan heterogeneity. They were further characterized after treatment with neuraminidase and endoproteases. All N-glycosylation sites exhibited fucose-contg. complex-type glycans. The N-glycosylation sites at Asn38 and Asn83 are mainly occupied by tetraantennary glycans, whereas Asn24 is occupied by a mixt. of bi-, tri- and tetraantennary glycans. A mol. mass glycoprofile for each glycosylation site was established based on the relative peak intensities obsd. in the MALDI mass spectra of the desialylated glycopeptides.
ST **erythropoietin** glycosylation site MALDI mass spectrometry
IT Protein motifs
(glycosylation site; glycopeptide profiling of human urinary **erythropoietin** by matrix-assisted laser desorption/ionization mass spectrometry)
IT Laser ionization mass spectrometry
(photodesorption, matrix-assisted; glycopeptide profiling of human urinary **erythropoietin** by matrix-assisted laser desorption/ionization mass spectrometry)
IT Laser desorption mass spectrometry
(photoionization, matrix-assisted; glycopeptide profiling of human urinary **erythropoietin** by matrix-assisted laser desorption/ionization mass spectrometry)
IT **11096-26-7, Erythropoietin**
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(human; glycopeptide profiling of human urinary **erythropoietin** by matrix-assisted laser desorption/ionization mass spectrometry)

L60 ANSWER 14 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1997:444395 HCAPLUS
DN 127:187341
TI Discovery of the shortest sequence motif for high level mucin-type O-glycosylation
AU Yoshida, Aruto; Suzuki, Misa; Ikenaga, Hiroshi; Takeuchi, Makoto
CS Central Lab. Key Technol., Kirin Brewery Co., Ltd., Kanazawa, 236, Japan
SO Journal of Biological Chemistry (1997), 272(27), 16884-16888
CODEN: JBCHA3; ISSN: 0021-9258
PB American Society for Biochemistry and Molecular Biology
DT Journal
LA English
CC 7-3 (Enzymes)
AB The census primary amino acid sequence for mucin-type O-**glycosylation** sites has not been identified. To det. the shortest motif sequence required for high level mucin-type O-**glycosylation**, we prepd. more than 100 synthetic peptides and assayed in vitro O-Gal-NAc transfer to serine or threonine in these peptides using a bovine colostrum UDP-N-**acetylgalactosamine**: polypeptide N-**acetylgalactosaminyl** transferase (O-GalNAcT). We chose the sequence PDAASAAP from human **erythropoietin** (hEPO) for further systematic substitutions because it accepted GalNAc and was a fairly simple sequence consisting only of four kinds of amino acids. Several substitutions showed that threonine is .apprx.40-fold better than serine as the **glycosylated** amino acid and a proline at position +3 on the C-terminal side is very important. To define the effect of proline residues around the **glycosylation** site, we analyzed a series of peptides contg. one to three proline residues in a parent peptides contg. one to three proline residues in a parent peptide AAATAAA. The results clearly indicated that prolines at positions +1 and +3 had a pos. effect. The O-GalNAc transfer level of AAATPAP was increased approx. 90-fold from AAATAAA. The deletion of amino acids from the N-terminal side of the **glycosylation** site suggested that five amino acids from position -1 to +3 were esp. important for **glycosylation**. Moreover, the influence of all 20 amino acids at positions -1, +2, and +4 was analyzed. Uncharged amino acids were preferred at position -1, and small or pos. charged amino acids were preferred at position +2. No preference was obsd. at position +4. We propose a mucin-type O-**glycosylation** motif, XTPXP, which may be suitable as a signal for protein O-**glycosylation**. The features obsd. in this study also appear to be very useful for prediction of mucin-type O-**glycosylation** sites in glycoproteins.
ST sequence motif mucin glycosylation site
IT **Glycosylation**
(**biol.**; shortest sequence motif for high level mucin-type O-glycosylation)
IT Structure-activity relationship
(enzyme substrate; shortest sequence motif for high level mucin-type O-glycosylation)
IT Protein motifs
(glycosylation site; shortest sequence motif for high level mucin-type O-glycosylation)
IT Mucins
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(shortest sequence motif for high level mucin-type O-glycosylation)
IT 9075-15-4
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)
(shortest sequence motif for high level mucin-type O-glycosylation)
IT 71524-43-1 149756-74-1 161148-36-3 178329-68-5 178329-69-6
178329-70-9 178329-71-0 178329-72-1 178329-73-2 178329-74-3
194363-86-5 194363-87-6 194363-88-7 194363-89-8 194363-90-1

194363-91-2 194363-92-3 194363-93-4 194363-94-5 194363-95-6
194363-96-7 194363-97-8 194363-98-9 194363-99-0 194364-00-6
194364-01-7 194364-02-8 194364-03-9 194364-04-0 194364-05-1
194364-06-2 194364-07-3 194364-08-4 194364-09-5 194364-10-8
194364-11-9 194364-12-0 194364-13-1 194364-14-2 194364-15-3
194364-16-4 194364-17-5 194364-18-6 194364-19-7 194364-20-0
194364-21-1 194364-22-2 194364-23-3 194364-24-4 194364-25-5
194364-26-6 194364-27-7 194364-28-8 194364-29-9 194364-30-2
194364-31-3 194364-32-4 194364-33-5 194364-34-6 194364-35-7
194364-36-8 194364-37-9 194364-38-0 194364-39-1 194364-40-4
194364-41-5 194364-42-6 194364-43-7 194364-44-8 194364-45-9
194364-46-0 194364-47-1 194364-48-2 194364-49-3 194364-50-6
194364-51-7 194364-52-8 194364-53-9 194364-54-0 194364-55-1
194364-56-2 194364-57-3 194364-58-4 194364-61-9 194364-63-1
194364-66-4
RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)
(shortest sequence motif for high level mucin-type O-glycosylation)

L60 ANSWER 15 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1997:226174 HCAPLUS
DN 126:334291
TI Microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide). Protein stability and the effects of stabilizing excipients
AU Morlock, Michael; **Koll, Hans**; Winter, Gerhard; Kissel, Thomas
CS Department Pharmaceutics Biopharmacy, Philipps University, Marburg, D-35032, Germany
SO European Journal of Pharmaceutics and Biopharmaceutics (1997), 43(1), 29-36
CODEN: EJPBEL; ISSN: 0939-6411
PB Elsevier
DT Journal
LA English
CC 63-5 (Pharmaceuticals)
Section cross-reference(s): 1
AB Parenteral delivery systems allowing controlled drug release over 1 mo are of particular interest for proteins and peptides. We investigated the microcapsulation of recombinant human **erythropoietin** (**EPO**), a stimulating factor of red blood cell prodn., into poly(D,L-lactide-co-glycolide) (PLG) microspheres, using a water-in-oil-in-water (W/O/W) double emulsion technique. The integrity and stability of **EPO** during microencapsulation and storage was characterized. Effects of excipients on in vitro release properties and formation of **EPO** aggregates were investigated. The formation of **EPO** aggregates in the W/O/W double emulsion technique was mainly influenced by the first homogenizing step, when prepg. the water-in-oil (W/O) emulsion, whereas the subsequent processing steps, including drying, proved to be noncrit. A rotor/stator homogenizer generated ca. 5% covalently bound **EPO** aggregates, ultrasonication and vortexing slightly increased aggregate-formation, as demonstrated by size-exclusion chromatog. and native-polyacrylamide gel electrophoresis. The discontinuous in vitro release behavior from PLG microspheres was not modified.
ST **erythropoietin** excipient microencapsulation biodegrdn protein peptide
IT Polymers, biological studies
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(biodegradable; microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))
IT Albumins, properties
RL: PRP (Properties)

(bovine; microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))
IT Drug delivery systems
(controlled-release; microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))
IT Aggregation
(inhibition; microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))
IT Drug delivery systems
(microcapsules; microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))
IT Encapsulation
(microencapsulation; microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))
IT 7585-39-9, .beta.-Cyclodextrin
RL: PRP (Properties)
(hydroxypropyl; microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))
IT 74-79-3, L-Arginine, properties
RL: PRP (Properties)
(microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))
IT 26780-50-7, Poly(D,L-lactide-co-glycolide)
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))
IT **11096-26-7, Erythropoietin**
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(recombinant human; microencapsulation of rh-**erythropoietin**, using biodegradable poly(D, L-lactide-co-glycolide))

L60 ANSWER 16 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1997:222328 HCAPLUS
DN 126:234148
TI Expression of human .alpha.2,6-sialyltransferase in BHK-21A cells increases the sialylation of coexpressed human **erythropoietin**: NeuAc-transfer onto GalNAc(.beta.1-4)GlcNAc-R motifs
AU Schlenke, Peter; Grabenhorst, Eckart; Wagner, Roland; Nimtz, Manfred; Conradt, Harald S.
CS Department of Gene Regulation and Differentiation, Braunschweig, D-38124, Germany
SO Animal Cell Technology: From Vaccines to Genetic Medicine, [Proceedings of the Meeting of the ESACT], 14th, Vilamoura, Port., May 1996 (1997), Meeting Date 1996, 475-480. Editor(s): Carrondo, Manuel J. T.; Griffiths, Bryan; Moreira, Jose L. P. Publisher: Kluwer, Dordrecht, Neth. CODEN: 64ELAL
DT Conference
LA English
CC 3-2 (Biochemical Genetics)
AB We have characterized two BHK cell lines (BHK-21B and BHK-21A) with different **glycosylation** properties. N-glycans synthesized by BHK-21B cells contain the typical N-**acetyllactosamine** motif (Gal(.beta.1-4)GlcNAc-R) whereas BHK-21A cells bear to a high amt. non-sialylated terminal GalNAc(.beta.1-4)GlcNAc-R moieties. Due to the incapability of the endogenous .alpha.2,3-sialyltransferase to transfer NeuAc to the GalNAc(.beta.1-4) GlcNAc-R structure recombinant glycoproteins produced by BHK-21A cells are under-sialylated. Therefore we have transfected BHK-21A cells harboring a plasmid encoding human **EPO** with the human Golgi enzyme CMP-NeuAc:Gal(.beta.1-4)GlcNAc-R .alpha.2,6-sialyltransferase (ST6N). Detailed structural anal. of oligosaccharides from the affinity purified recombinant **EPO**

(HPAEC-PAD-mapping and MALDI/TOF-MS) revealed a significant increased NeuAc content when compared to the parent BHK-21A cells without ST6N activity. Methylation anal. corroborated these results. The newly introduced .alpha.2,6-sialyltransferase recognizes the terminal GlcNAc-R motif as a substrate. The cell line obtained thus exhibits a "human kidney-type" **glycosylation** characteristic.

ST BHK cell human sialyltransferase **erythropoietin** sialylation

IT Animal cell line
(BHK-21A; expression of human .alpha.2,6-sialyltransferase in BHK-21A cells increases the sialylation of coexpressed human **erythropoietin**: NeuAc-transfer onto GalNAc(.beta.1-4)GlcNAc-R motifs)

IT **Sialylation**
(expression of human .alpha.2,6-sialyltransferase in BHK-21A cells increases the sialylation of coexpressed human **erythropoietin** : NeuAc-transfer onto GalNAc(.beta.1-4)GlcNAc-R motifs)

IT 9075-81-4, .alpha.2-6 Sialyltransferase
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(expression of human .alpha.2,6-sialyltransferase in BHK-21A cells increases the sialylation of coexpressed human **erythropoietin** : NeuAc-transfer onto GalNAc(.beta.1-4)GlcNAc-R motifs)

IT **11096-26-7, Erythropoietin**
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(expression of human .alpha.2,6-sialyltransferase in BHK-21A cells increases the sialylation of coexpressed human **erythropoietin** : NeuAc-transfer onto GalNAc(.beta.1-4)GlcNAc-R motifs)

L60 ANSWER 17 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1997:172455 HCAPLUS
DN 126:168439
TI A cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for the production of modified glycoproteins
IN Schauer, Roland; Schlenzka, Wiebke; Kelm, Soerge; Shaw, Lee; **Haselbeck, Anton**; Honold, Konrad
PA Boehringer Mannheim Gmbh, Germany
SO Eur. Pat. Appl., 24 pp.
CODEN: EPXXDW
DT Patent
LA English
IC ICM C12N015-53
ICS C12N009-02; C12Q001-68; C12P019-00; C07K014-505
CC 7-2 (Enzymes)
Section cross-reference(s): 3
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	EP 752474	A1	19970108	EP 1995-110609	19950707
	R: DE				
	WO 9703200	A1	19970130	WO 1996-EP2966	19960705
	W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA				
	AU 9666118	A1	19970210	AU 1996-66118	19960705
	EP 837942	A1	19980429	EP 1996-925669	19960705
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
	JP 10510434	T2	19981013	JP 1996-505487	19960705
PRAI	EP 1995-110609		19950707		

EP 1995-114930 19950922
WO 1996-EP2966 19960705

AB A cDNA for swine submandibular CMP-N-acetyl-neuraminic acid hydroxylase is cloned for use in the prepn. of the enzyme free of contamination with cytochrome b5 and cytochrome b5 reductase. The cDNA may be expressed in a prokaryotic or eukaryotic systems and it can be used in the construction of host cells for the manuf. of proteins lacking N-glycoloylneuraminic acid side chains, esp. **erythropoietin** and the colony-stimulating factors. Purifn. of the enzyme and cloning by PCR of a cDNA is described.

ST antisense DNA acetylneuraminic acid hydroxylase; acetylneuraminic acid hydroxylase pig cDNA; **erythropoietin** glycosidation acetylneuraminic acid hydroxylase

IT Swine
(cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT Gene, animal
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); USES (Uses)
(cDNA, for pig CMP-N-acetyl-neuraminic acid hydroxylase; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT cDNA sequences
(for CMP-N-acetyl-neuraminic acid hydroxylase of pig; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT Glycoproteins, general, biological studies
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
(glycosidation of; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT Antisense DNA
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(inhibiting expression of CMP-N-acetyl-neuraminic acid hydroxylase gene; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT Probes (nucleic acid)
RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)
(labeled, for detection of CMP-N-acetyl-neuraminic acid hydroxylase gene expression; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT Protein sequences
(of CMP-N-acetyl-neuraminic acid hydroxylase of pig; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT Transformation, genetic
(of animal cells with antisense DNA to CMP-N-acetyl-neuraminic acid hydroxylase; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT Glycosylation
(of proteins manufd. in transgenic hosts, control of; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT Salivary gland
(submandibular, CMP-N-acetyl-neuraminic acid hydroxylase of; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT 187043-51-2P
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)

(amino acid sequence, cloning and expression of cDNA for; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT **11096-26-7P, Erythropoietin**
RL: BPN (Biosynthetic preparation); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); PREP (Preparation)
(biosynthesis and glycosidation in transgenic cells of; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT 116036-67-0P, Cmp-n-acetyl-neuraminic acid hydroxylase
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation); USES (Uses)
(cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT 1113-83-3D, N-Glycoloylneuraminic acid, oligosaccharides contg.
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(glycoproteins free of, prepn. of; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT 187043-46-5 187043-47-6
RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)
(nucleotide sequence, cloning and expression of; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT 9032-25-1, Cytochrome b5 reductase 9035-39-6, Cytochrome b5
RL: MSC (Miscellaneous)
(pig CMP-N-acetyl-neuraminic acid hydroxylase free of, prepn. of; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

IT 25104-18-1D, Polylysine, conjugates with antisense oligonucleotides
38000-06-5D, Polylysine, conjugates with antisense oligonucleotides
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(transformation of animal cells with; cDNA for pig CMP-N-acetyl-neuraminic acid hydroxylase and its use for prodn. of modified glycoproteins)

L60 ANSWER 18 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1997:38803 HCAPLUS
DN 126:57095
TI Human **erythropoietin** recombinant production by fermentation and protein purification using a series of chromatographic steps
IN **Burg, Josef**; Schneider, Walter; Wrba, Alexander; Fuerst, Werner; **Sellinger, Karl-Heinz**
PA Boehringer Mannheim Gmbh, Germany; Burg, Josef; Schneider, Walter; Wrba, Alexander; Fuerst, Werner; Sellinger, Karl-Heinz
SO PCT Int. Appl., 25 pp.
CODEN: PIXXD2
DT Patent
LA German
IC ICM C07K014-505
ICS A61K038-18
CC 9-3 (Biochemical Methods)
Section cross-reference(s): 3, 13
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9635718	A1	19961114	WO 1996-EP1988	19960510

W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT,

LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN

CA	2220515	AA	19961114	CA 1996-2220515	19960510
AU	9658174	A1	19961129	AU 1996-58174	19960510
ZA	9603713	A	19971110	ZA 1996-3713	19960510
EP	830376	A1	19980325	EP 1996-919753	19960510
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
JP	10506924	T2	19980707	JP 1996-533775	19960510
JP	3061200	B2	20000710		
US	6399333	B1	20020604	US 1998-945802	19980213
US	2002146771	A1	20021010	US 2002-67235	20020207
US	6471500	B2	20021029		
PRAI EP	1995-107165	A	19950511		
DE	1995-19522461	A	19950621		
WO	1996-EP1988	W	19960510		
US	1998-945802	A1	19980213		

AB A prepn. of a protein with **erythropoietin** activity and obtainable after culturing an **erythropoietin**-producing host cell is characterized by a host-cell proteins content of .ltoreq. 100 ppm, a host cell DNA content of .ltoreq. 10 pg per 83 .mu.g of **erythropoietin**, and a total absence of mammalian proteins not derived from the host cell. The prepn. is obtained following serum-free culture using a purifn. process involving dye chromatog., hydrophobic chromatog. on an alkylated or arylated carrier, chromatog. on hydroxyapatite, hydrophobic chromatog. and anion exchange chromatog.

ST **erythropoietin** prodn fermn chromatog purifn

IT Affinity chromatography
(Blue Sepharose or other dye affinity chromatog.; human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT Hydrophobic interaction chromatography
(Bu Toyopearl or other alkylated or arylated carrier hydrophobic chromatog.; human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT Animal cell line
(CHO, host cell; human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT Alcohols, uses
RL: NUU (Other use, unclassified); USES (Uses)
(chromatog. eluent; human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT Eukaryote (Eukaryotae)
(host cell; human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT Anion exchange liquid chromatography
Fermentation
Reversed phase HPLC
(human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT Gene, animal
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)
(human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT 75432-66-5
RL: NUU (Other use, unclassified); USES (Uses)
(Blue Sepharose or other dye affinity chromatog.; human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT 67-63-0, Isopropanol, uses

RL: NUU (Other use, unclassified); USES (Uses)
(chromatog. eluent; human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT 1306-06-5, Hydroxyapatite 78922-65-3, Ultrogel 133876-61-6, DEAE Sepharose fast flow
RL: NUU (Other use, unclassified); USES (Uses)
(chromatog.; human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

IT **11096-26-7P, Erythropoietin**
RL: BMF (Bioindustrial manufacture); PUR (Purification or recovery); BIOL (Biological study); PREP (Preparation)
(human **erythropoietin** recombinant prodn. by fermn. and protein purifn. using series of chromatog. steps)

L60 ANSWER 19 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1996:701504 HCAPLUS
DN 125:339026
TI Polypeptide-containing pharmaceutical forms of administration in the form of microparticles
IN **Koll, Hans**; Winter, Gerhard; Kissel, Thomas; Morlock, Michael
PA Boehringer Mannheim Gmbh, Germany
SO PCT Int. Appl., 30 pp.
CODEN: PIXXD2
DT Patent
LA German
IC ICM A61K009-16
ICS A61K009-50
CC 63-6 (Pharmaceuticals)
FAN.CNT 2

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9628143	A1	19960919	WO 1996-EP980	19960307
	W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI				
	RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN				
	DE 19513659	A1	19960912	DE 1995-19513659	19950411
	DE 19542837	A1	19970522	DE 1995-19542837	19951117
	AU 9650041	A1	19961002	AU 1996-50041	19960307
	EP 814778	A1	19980107	EP 1996-906751	19960307
	EP 814778	B1	20010613		
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
	JP 11501642	T2	19990209	JP 1996-527251	19960307
	US 6346274	B1	20020212	US 1998-894796	19980105
PRAI	DE 1995-19508612	A	19950310		
	DE 1995-19513659	A	19950411		
	DE 1995-19542837	A	19951117		
	WO 1996-EP980	W	19960307		

AB The title microparticles contain as biodegradable polymer an ABA-triblock copolymer whose A-block is a lactic and glycolic acid copolymer and whose B-block is a polyethylene glycol chain, together with additives selected from serum proteins, polyamino acids, cyclodextrins, cyclodextrin derivs., saccharides, amino sugars, amino acids, detergents, or carboxylic acids and mixts. of these additives. The microparticles continuously release the polypeptide over a relatively long period of time even when the amts. of polypeptide they include are small or sensitive to aggregation. Thus, 700 mg ABA copolymer of the above type (DL-lactic acid:glycolic acid:PEG ratio = 50:12:38) was dissolved in 2.5 mL CH_2Cl_2, homogenized with a soln. of 3.5 mg **erythropoietin** (and optionally additives) in 0.8 mL H_2O, and then homogenized with 300 mL 0.1% aq. PVA soln. to produce a water-in-oil-in-water emulsion; the CH_2Cl_2 phase was then removed by

evapn. to produce microparticles. The **erythropoietin** was 10-20% aggregated in the absence of additives; aggregation was markedly diminished by addn. of e.g. 5-10 wt.% bovine serum albumin.
ST polypeptide pharmaceutical microparticle biodegradable polymer; lactate glycolate PEG copolymer protein microparticle
IT Albumins, biological studies
Amino acids, biological studies
Carbohydrates and Sugars, biological studies
Carboxylic acids, biological studies
Detergents
Glycerides, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(aggregation inhibitors; polypeptide-contg. pharmaceutical microparticles)
IT Agglomeration preventers
(polypeptide-contg. pharmaceutical microparticles)
IT Proteins, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(polypeptide-contg. pharmaceutical microparticles)
IT Carbohydrates and Sugars, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(aminodeoxy, aggregation inhibitors; polypeptide-contg. pharmaceutical microparticles)
IT Pharmaceutical dosage forms
(microparticles, polypeptide-contg. pharmaceutical microparticles)
IT Polyamides, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(poly(amino acids), aggregation inhibitors; polypeptide-contg. pharmaceutical microparticles)
IT 56-40-6, Glycine, biological studies 56-86-0, Glutamic acid, biological studies 56-87-1, L-Lysine, biological studies 57-50-1, biological studies 63-91-2, Phenylalanine, biological studies 69-79-4 74-79-3, Arginine, biological studies 99-20-7, Trehalose 512-69-6 9004-54-0, Dextran, biological studies 9005-25-8, Starch, biological studies 9005-32-7, Alginic acid 9005-64-5, Tween 20 9005-65-6, Tween 80 9012-76-4, Chitosan 9050-36-6, Maltodextrin 12619-70-4, Cyclodextrin 12619-70-4D, Cyclodextrin, 2-hydroxypropyl ethers 24937-47-1, Polyarginine 25212-18-4, Polyarginine 26062-48-6, Polyhistidine 26854-81-9, Polyhistidine 106392-12-5, Pluronic F68
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(aggregation inhibitor; polypeptide-contg. pharmaceutical microparticles)
IT 12619-70-4D, Cyclodextrin, derivs.
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(aggregation inhibitors; polypeptide-contg. pharmaceutical microparticles)
IT **11096-26-7, Erythropoietin**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(polypeptide-contg. pharmaceutical microparticles)
IT 164584-68-3
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(triblock; polypeptide-contg. pharmaceutical microparticles)

L60 ANSWER 20 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1996:672519 HCAPLUS
DN 125:309023
TI Polypeptide-containing pharmaceutical dosage form comprising microparticles
IN Winter, Gerhard; **Koll, Hans**; Kissel, Thomas; Morlock, Michael

PA Boehringer Mannheim GmbH, Germany
SO Ger. Offen., 10 pp.
CODEN: GWXXBX
DT Patent
LA German
IC ICM A61K009-50
ICS A61K038-00; A61K047-26; A61K047-40; A61K047-42; A61K047-12; A61K047-30
CC 63-6 (Pharmaceuticals)
FAN.CNT 2

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI DE 19513659	A1	19960912	DE 1995-19513659	19950411
CA 2214889	AA	19960919	CA 1996-2214889	19960307
WO 9628143	A1	19960919	WO 1996-EP980	19960307
W: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN				
AU 9650041	A1	19961002	AU 1996-50041	19960307
EP 814778	A1	19980107	EP 1996-906751	19960307
EP 814778	B1	20010613		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, PT, IE, FI				
JP 11501642	T2	19990209	JP 1996-527251	19960307
ES 2159726	T3	20011016	ES 1996-906751	19960307
US 6346274	B1	20020212	US 1998-894796	19980105
PRAI DE 1995-19508612	A1	19950310		
DE 1995-19513659	A	19950411		
DE 1995-19542837	A	19951117		
WO 1996-EP980	W	19960307		

AB A polypeptide drug is administered in the form of microparticles having a matrix of a biodegradable ABA triblock copolymer (A = lactic acid/glycolic acid copolymer; B = PEG) which addnl. contains excipients selected from cyclodextrin, cyclodextrin derivs., saccharides, amino sugars, amino acids, polyamino acids, and serum proteins. These microparticles provide sustained release of the polypeptide while stabilizing the polypeptide by preventing its aggregation. Thus, 709 mg ABA block copolymer was dissolved in 2.33 mL CH2Cl2 and homogenized with 0.8 mL buffered aq. soln. contg. 3.5 mg **erythropoietin**, 5% dextran, and 1% polyarginine. This mixt. was then homogenized with 300 mL 0.1% aq. PVA soln. to produce a water-in-oil-in-water emulsion, the CH2Cl2 phase was evapd., and the microparticles were collected by filtration, washed, and lyophilized. The degree of aggregation of **erythropoietin** extd. from the particles was only 10%, compared to 28% from particles not contg. dextran and polyarginine.

ST polypeptide drug microparticle lactate glycolate copolymer; **erythropoietin** microparticle sustained release

IT Agglomeration preventers
(polypeptide-contg. pharmaceutical dosage form comprising microparticles)

IT Proteins, biological studies
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(polypeptide-contg. pharmaceutical dosage form comprising microparticles)

IT Albumins, biological studies
Amino acids, biological studies
Carbohydrates and Sugars, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(polypeptide-contg. pharmaceutical dosage form comprising

microparticles)
IT Molecular association
(prevention of; polypeptide-contg. pharmaceutical dosage form comprising microparticles)
IT Carbohydrates and Sugars, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(aminodeoxy, polypeptide-contg. pharmaceutical dosage form comprising microparticles)
IT Pharmaceutical dosage forms
(microparticles, polypeptide-contg. pharmaceutical dosage form comprising microparticles)
IT Polyamides, biological studies
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(poly(amino acids), polypeptide-contg. pharmaceutical dosage form comprising microparticles)
IT **11096-26-7, Erythropoietin**
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(polypeptide-contg. pharmaceutical dosage form comprising microparticles)
IT 56-40-6, Glycine, biological studies 56-86-0, Glutamic acid, biological studies 56-87-1, Lysine, biological studies 57-50-1, Sucrose, biological studies 63-91-2, Phenylalanine, biological studies 69-79-4, Maltose 74-79-3, Arginine, biological studies 99-20-7, Trehalose 512-69-6, Raffinose 9004-54-0, Dextran, biological studies 9005-25-8, Starch, biological studies 9005-32-7, Alginic acid 9012-76-4, Chitosan 9050-36-6, Maltodextrin 12619-70-4, Cyclodextrin 12619-70-4D, Cyclodextrin, 2-hydroxypropyl ethers 12619-70-4D, Cyclodextrin, derivs. 24937-47-1, Polyarginine 25212-18-4, Polyarginine 26062-48-6, Polyhistidine 26854-81-9, Polyhistidine
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(polypeptide-contg. pharmaceutical dosage form comprising microparticles)
IT 154326-36-0
RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
(triblock; polypeptide-contg. pharmaceutical dosage form comprising microparticles)

L60 ANSWER 21 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN **1996:600168** HCAPLUS
DN **125:293261**
TI Lectin-binding assays for the isoforms of human **erythropoietin**: comparison of urinary and four recombinant **erythropoietins**
AU Storring, P. L.; Tiplady, R. J.; Gaines Das, R. E.; Rafferty, B.; Mistry, Y. G.
CS National Inst. Biol. Stds. Control, Hertfordshire, EN6 3QG, UK
SO Journal of Endocrinology (1996), 150(3), 401-412
CODEN: JOENAK; ISSN: 0022-0795
PB Journal of Endocrinology
DT Journal
LA English
CC 2-1 (Mammalian Hormones)
AB Assays have been developed for the isoforms of **erythropoietin** (**EPO**) based on their binding to eight different lectins. These assays were used to compare the isoform compns. of two prepns. of human urinary **EPO** (uEPO) and four prepns. of recombinant DNA-derived human **EPO** (rEPO), which had been shown to differ in their biol. and immunol. properties and in their isoform compn. as judged by isoelec. focusing and electrophoresis. Agarose-bound Ricinus communis agglutinin I (RCA), Erythrina cristagalli agglutinin (ECA), Maackia amurensis leucoagglutinin (MAL), Sambucus nigra agglutinin (SNA), Lycopersicon esculentum agglutinin (LEA), Con A, Phaseolus vulgaris agglutinin-L4

(L-PHA) and Agaricus bisporus agglutinin (ABA) were used to bind **EPO** isoforms possessing: N-glycans contg. non-sialylated outer Gal.beta.1-4GlcNAc (RCA and ECA), NeuAc.alpha.2-3Gal.beta.1-4GlcNAc (MAL), NeuAc.alpha.2-6-Gal (SNA), or repeating Gal.beta.1-4GlcNAc sequences (LEA); biantennary N-glycans (Con A); tetraantennary and 2,6-branched triantennary N-glycans (L-PHA); and O-glycans contg. NeuAc.alpha.2-6GalNAc (SNA) and Gal.beta.1-3GalNAc (ABA). Free **EPO** was measured by mouse spleen cell bioassay or immunoassay. Ests. from most lectin-binding assays were reproducible between assays and batches of lectin-agarose, although batches of MAL- and ABA-agarose, and to a lesser extent LEA-agarose, differed in their EP-binding. Lectin-binding assays showed differences between the isoform compns. of all **EPOs**, including the two Chinese hamster ovary cell-derived rEPOs, with RCA- and ECA-binding assays being the most discriminating. Lectin-binding ests. provided evidence that uEPO differs from these rEPOs in its lower content of isoforms with biantennary N-glycans and higher content of those with multiantennary N-glycans, and in its lower content of isoforms with N-glycans possessing repeating Gal.beta.1-4GlcNAc sequences and of those with O-glycans contg. Gal.beta.1-3GalNAc. Lectin-binding ests. also indicated that, contrary to some reports, uEPO possesses Gal.beta.1-3GalNAc-contg. O-glycans but not Neu-Ac.alpha.2-6GalNAc-contg. O-glycans or NeuAc.alpha.2-6Gal-contg. N-glycans. Most groups of lectin-bound **EPO** isoforms did not differ in their relative bioactivities and immunoreactivities. Most groups of lectin-bound **EPO** isoforms did not differ in their relative bioactivities and immunoreactivities. However, ests. for ABA-bound **EPO** isoforms suggested that O-glycans might influence the bioactivity of **EPO** differently to its immunoreactivity. Furthermore, the bioactivities of some ECA-bound **EPO** isoforms were higher, and those of some of the MAL-bound **EPO** isoforms lower, than their immunoreactivities, consistent with the reported enhancement of **EPO** in vitro bioactivity by desialylation.

ST lectin binding **erythropoietin** isoform

IT Agglutinins and Lectins
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(lectin-binding assays for the isoforms of human **erythropoietin** for the comparison of urinary and four recombinant **erythropoietins**)

IT **11096-26-7, Erythropoietin**
RL: ANT (Analyte); ANST (Analytical study)
(isoforms; lectin-binding assays for the isoforms of human **erythropoietin** for the comparison of urinary and four recombinant **erythropoietins**)

IT 3554-90-3D, Gal.beta.1-3GalNAc, lectin-contg. **32181-59-2D**, Gal.beta.1-4GlcNAc, lectin-contg. 35259-23-5D, NeuAc.alpha.2-6-Gal, lectin-contg. 72506-87-7D, NeuAc.alpha.2-6GalNAc, lectin-contg. 81693-22-3D, NeuAc.alpha.2-3Gal.beta.1-4GlcNAc, lectin-contg.
RL: BUU (Biological use, unclassified); BIOL (Biological study); USES (Uses)
(lectin-binding assays for the isoforms of human **erythropoietin** for the comparison of urinary and four recombinant **erythropoietins**)

L60 ANSWER 22 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1996:437103 HCAPLUS
DN 125:105333
TI Carbohydrate structure of N- and O-linked oligosaccharides of human **erythropoietin** expressed in Chinese hamster ovary cells
AU Lee, Dong Eok; Ha, Byung Jhip; Kim, Suk Joon; Park, Ji Sook; Yoo, Ree Ann; Oh, Myung Suk; Kim, Hyun Su
CS R&D center, Cheil Foods & Chemicals Inc., Kyonggi-do, 467-810, S. Korea
SO Journal of Biochemistry and Molecular Biology (1996), 29(3), 266-271

CODEN: JBMBE5; ISSN: 1225-8687
PB Biochemical Society of the Republic of Korea .
DT Journal
LA English
CC 2-2 (Mammalian Hormones)
AB A recombinant human **erythropoietin** (**EPO**), expressed in Chinese hamster ovary (CHO) cells, is **glycosylated** at Asn 24, Asn 38, Asn 83, and Ser 126. After release of the N-linked carbohydrate chains by peptide-N4-(N-**acetyl**-.beta.-glucosaminyl)asparagine amidase F, the oligosaccharides were analyzed by FACE (Fluorophore-Assisted Carbohydrate Electrophoresis). The O-linked carbohydrate chain was sepd. by hydrazine, and analyzed by FACE. The monosaccharide compn. of recombinant **EPO** showed mannose, fucose, galactose, N-**acetylglucosamine**, N-**acetylneuraminic** acid, and a trace of N-**acetylgalactosamine**, which are typical monosaccharides in the glycoproteins from the CHO cell. Sequences of N-linked and O-linked oligosaccharides were detd. The structure and compn. of oligosaccharides attached to recombinant human **EPO**, expressed in the CHO cell, are identical to the reported oligosaccharides attached to recombinant human **EPO**, expressed in the CHO cell, are identical to the reported oligosaccharide structure in human **EPO** isolated from urine.
ST carbohydrate structure **erythropoietin**; oligosaccharide structure **erythropoietin**
IT **Glycosidation**
Molecular structure, natural product
(carbohydrate structure of N- and O-linked oligosaccharides of human **erythropoietin** expressed in Chinese hamster ovary cells)
IT Carbohydrates and Sugars, biological studies
Oligosaccharides
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(carbohydrate structure of N- and O-linked oligosaccharides of human **erythropoietin** expressed in Chinese hamster ovary cells)
IT 59-23-4, D-Galactose, biological studies 131-48-6, N-Acetylneuraminic acid 1811-31-0, N-**Acetylgalactosamine** 2438-80-4, L-Fucose 3458-28-4, D-Mannose 7512-17-6, N-Acetylglucosamine
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(carbohydrate structure of N- and O-linked oligosaccharides of human **erythropoietin** expressed in Chinese hamster ovary cells)
IT **11096-26-7, Erythropoietin**
RL: PRP (Properties)
(carbohydrate structure of N- and O-linked oligosaccharides of human **erythropoietin** expressed in Chinese hamster ovary cells)
IT 70-47-3, Asparagine, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(human **erythropoietin** asparagine residues 24 and 38 and 83 as glycosidation sites)
IT 56-45-1, Serine, biological studies
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)
(human **erythropoietin** serine residue 126 as glycosidation site)

L60 ANSWER 23 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1995:479977 HCAPLUS
DN 124:45903
TI Structural analysis of the sialylated N- and O-linked carbohydrate chains of recombinant human **erythropoietin** expressed in Chinese hamster ovary cells. Sialylation patterns and branch location of dimeric N-**acetyllactosamine** units

AU Hokke, Cornelis H.; Bergwerff, Aldert A.; Van Dedem, Gijs W. K.; Kamerling, Johannis P.; Vliegenthart, Johannes F. G.
CS Department Bio-Organic Chemistry, Utrecht University, Neth.
SO European Journal of Biochemistry (1995), 228(3), 981-1008
CODEN: EJBCAI; ISSN: 0014-2956
PB Springer
DT Journal
LA English
CC 2-2 (Mammalian Hormones)
AB The N-linked carbohydrate chains of recombinant human **erythropoietin** expressed in CHO cells were quant. released with peptide-N4-(N-**acetyl**-.beta.-glucosaminyl)asparagine amidase F, sepd. from the remaining O-glucoprotein by gel-permeation chromatog., and subsequently fractionated via FPLC on Mono Q, HPLC on Lichrosorb-NH2 and high-pH anion-exchange chromatog. on CarboPac PA1. The purified sialylated oligosaccharides were analyzed by one-dimensional and two-dimensional 500-MHz 1H-NMR spectroscopy. When necessary, oligosaccharides were treated with endo-.beta.-galactosidase (and N-**acetyl**-.beta.-glucosaminidase) followed by 1H-NMR anal. of the incubation products, to obtain addnl. structural information. Di-, tri-, tri'- and tetraantennary N-**acetyllactosamine**-type oligosaccharides occur which can be completely (major) or partially (minor) sialylated. Three different types of .alpha.2-3-linked sialic acids are present, namely, N-**acetylneuraminic** acid (95%), N-glycolylneuraminic acid (2%) and N-**acetyl**-9-O-**acetylneuraminic** acid (3%). In the case of partial sialylation, a non-random distribution of the sialic acids over the branches is obsd. One or two extra N-**acetyllactosamine** units, being exclusively located in the branches attached to the .alpha.1-6-linked Man residue, can be present in completely or partially sialylated di-, tri'-, and tetraantennary oligosaccharides. Tetraantennary oligosaccharides with N-**acetyllactosamine** repeats could be digested quant. with endo-.beta.-galactosidase from Bacteroides fragilis, whereas under the same conditions tri' antennary oligosaccharides hardly reacted (<15%). Using endo-.beta.-galactosidase from Escherichia freundii, these triantennary oligosaccharides could be digested more extensively (>75%). The O-linked carbohydrate chains were released from the O-glycoprotein by alk. borohydride treatment, and purified via FPLC on Mono Q and HPLC on Lichrosorb-NH2. Two O-glycans were found, namely, Neu5Ac.alpha.2-3Gal.beta.1-3GalNAc-ol and Neu5Ac.alpha.2-3Gal.beta.1-3(Neu5Ac.alpha.2-6)GalNAc-ol.
ST carbohydrate structure **erythropoietin** sialylation **acetyllactosamine**
IT Molecular structure, natural product
(sialylation patterns and branch location of dimeric N-**acetyllactosamine** units in recombinant human **erythropoietin** expressed in Chinese hamster ovary cells)
IT Carbohydrates and Sugars, properties
Oligosaccharides
Sialic acids
RL: PRP (Properties)
(sialylation patterns and branch location of dimeric N-**acetyllactosamine** units in recombinant human **erythropoietin** expressed in Chinese hamster ovary cells)
IT **Glycosidation**
(**sialylation, sialylation** patterns and branch location of dimeric N-**acetyllactosamine** units in recombinant human **erythropoietin** expressed in Chinese hamster ovary cells)
IT **11096-26-7, Erythropoietin**
RL: PRP (Properties)
(recombinant human; sialylation patterns and branch location of dimeric N-**acetyllactosamine** units in recombinant human

erythropoietin expressed in Chinese hamster ovary cells)

IT 93375-83-8 113799-69-2 118352-68-4 118352-69-5 121283-16-7
123430-80-8 137366-59-7 137366-60-0 148555-24-2 148943-61-7
148943-62-8 162557-23-5 162557-24-6 162557-25-7 162557-26-8
162557-27-9 162557-28-0 162557-29-1 162557-30-4 162557-31-5
162557-32-6 162557-33-7 162557-34-8 162627-53-4 162627-54-5
162627-55-6 162627-56-7 162627-57-8 162627-58-9 162627-66-9
RL: PRP (Properties)
(sialylation patterns and branch location of dimeric N-**acetyllactosamine** units in recombinant human **erythropoietin** expressed in Chinese hamster ovary cells)

L60 ANSWER 24 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1995:448553 HCAPLUS
DN 123:2184
TI Microheterogeneity of **Erythropoietin** Carbohydrate Structure
AU Rush, Robert S.; Derby, Patricia L.; Smith, Duncan M.; Merry, Catherine; Rogers, Gary; Rohde, Michael F.; Katta, Viswanatham
CS Department of Protein Structure, AMGEN Inc., Thousand Oaks, CA, 91320-1789, USA
SO Analytical Chemistry (1995), 67(8), 1442-52
CODEN: ANCHAM; ISSN: 0003-2700
PB American Chemical Society
DT Journal
LA English
CC 3-2 (Biochemical Genetics)
Section cross-reference(s): 2, 9
AB The microheterogeneity of the carbohydrate structures on recombinant human **erythropoietin** (rHuEPO) expressed in Chinese hamster ovary cells has been evaluated by electrospray ionization (ESI) mass spectrometry (MS) of glycopeptide fragments. The microheterogeneity is largely assocd. with the presence or absence of terminal N-**acetylneuraminic** acid (Neu5Ac) residues, varying amts. of O-**acetylation** of the Neu5Ac residues, and the presence or absence of N-**acetyllactosamine** extensions. The N-linked carbohydrate structures were structurally diverse; 52 different N-linked oligosaccharide structures were identified. Consistent structural assignments could be made from data obtained using different proteolytic digests, ESI solvent systems (aq./methanol systems with acetic or formic acid), and online or off-line LC/MS anal. All **glycosylation** sites exhibited some level of O-**acetylation** of Neu5Ac residues. Interestingly, **glycosylation** site asparagine-83 exhibits mono-O-**acetyl** and di-O-**acetyl** Neu5Ac residues, while the other sites, asparagine-24, asparagine-38, and serine-126, exhibit mainly mono-O-**acetyl** Neu5Ac derivatization. This difference in O-**acetylation** may be site specific or due to sample handling of labile structures. However, mild base treatment of rHuEPO with NaOH on ice removed the O-**acetyl** groups assocd. with a given carbohydrate structure, without adversely affecting the underlying oligosaccharide structure, resulting in a simplified mass spectra. NMR spectroscopy of Neu5Ac residues released by neuraminidase treatment of total rHuEPO indicated that Neu5,9Ac2 residues were present. Addnl. resonances were also obsd. that were consistent with other Neu5Ac O-**acetyl** linkages; these O-**acetyl** resonances could be removed by mild base hydrolysis of rHuEPO.
ST human recombinant **erythropoietin** carbohydrate microheterogeneity
IT Acetylation
(microheterogeneity of recombinant human **erythropoietin** carbohydrate structure)
IT Oligosaccharides
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence)
(microheterogeneity of recombinant human **erythropoietin** carbohydrate structure)

IT Animal tissue culture
(microheterogeneity of recombinant human **erythropoietin** carbohydrate structure produced by)
IT Animal cell line
(CHO, microheterogeneity of recombinant human **erythropoietin** carbohydrate structure from)
IT 131-48-6, N-Acetylneuraminic acid **32181-59-2**, N-**Acetyllactosamine** 55717-54-9
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence)
(microheterogeneity of recombinant human **erythropoietin** carbohydrate structure)
IT **11096-26-7, Erythropoietin**
RL: PRP (Properties)
(microheterogeneity of recombinant human **erythropoietin** carbohydrate structure)

L60 ANSWER 25 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1994:551510 HCAPLUS
DN 121:151510
TI A comparison of serine and threonine O-glycosylation by UDP-GalNAc:polypeptide N-**acetylgalactosaminyltransferase**
AU O'Connell, B.C.; Tabak, L.A.
CS Dep. Dent. Res. Biochem., Univ. Rochester, Rochester, NY, 14642, USA
SO Journal of Dental Research (1993), 72(12), 1554-8
CODEN: JDREAF; ISSN: 0022-0345
DT Journal
LA English
CC 6-3 (General Biochemistry)
Section cross-reference(s): 7
AB O-**glycosylated** proteins are ubiquitous in eukaryotes and are responsible for a variety of biol. functions. O-**glycosylation** is initiated by the addn. of N-**acetylgalactosamine** to serine or threonine residues, though it is not clear how specific residues are selected for modification. The authors have compared serine and threonine **glycosylation** using peptide substrates based on sequences from **erythropoietin** (**EPO**) and von Willebrand factor (HVF) that are **glycosylated** in vivo. UDP-GalNAc:-polypeptide N-**acetylgalactosaminyltransferase** was derived from rat parotid, submandibular, and sublingual glands, liver and kidney as well as from human colostrum. The threonine-contg. substrates were **glycosylated** to a much greater extent than those contg. serine for all the enzyme sources. Changes in reaction pH, donor concn., or divalent cation were unable to increase **glycosylation** of serine. When the incubation time was extended, serine in the **EPO**-based peptide was found to incorporate GalNAc at a low level, in contrast to the serine-contg. HVF peptide, which did not **glycosylate** at all. By CD, the non-**glycosylating** peptide was the only one of the series that did not exhibit random coil structure. The authors' data suggest that although the structural and sequence requirements for O-**glycosylation** of serine and threonine residues are similar, serine sites are **glycosylated** less effectively than are threonine sites in vitro.
ST protein serine threonine glycosylation **acetylgalactosaminyltransferase**
IT Kidney
Liver
(protein **acetylgalactosaminyltransferase** of, **erythropoietin** and von Willebrand factor peptides serine and threonine residues **glycosylation** by)
IT Colostrum
(protein **acetylgalactosaminyltransferase** of, of human, **erythropoietin** and von Willebrand factor peptides serine and

threonine residues **glycosylation** by)
IT Salivary gland
(parotid, protein **acetylgalactosaminyltransferase** of, **erythropoietin** and von Willebrand factor peptides serine and threonine residues **glycosylation** by)
IT Conformation and Conformers
(secondary, of serine- and threonine-contg. peptides, glycosylation by protein **acetylgalactosaminyltransferase** in relation to)
IT Salivary gland
(sublingual, protein **acetylgalactosaminyltransferase** of, **erythropoietin** and von Willebrand factor peptides serine and threonine residues **glycosylation** by)
IT Salivary gland
(submandibular, protein **acetylgalactosaminyltransferase** of, **erythropoietin** and von Willebrand factor peptides serine and threonine residues **glycosylation** by)
IT **11096-26-7, Erythropoietin** 109319-16-6, Von Willebrand factor
RL: BIOL (Biological study)
(**glycosylation** of serine and threonine residues of, by protein **acetylgalactosaminyltransferase**)
IT 9075-15-4, UDP-**acetylgalactosamine**-glycoprotein **acetylgalactosaminyltransferase**
RL: BIOL (Biological study)
(serine and threonine residues O-glycosylation by, factors in)

L60 ANSWER 26 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1993:576469 HCAPLUS
DN 119:176469
TI The acceptor substrate specificity of porcine submaxillary UDP-GalNAc:polypeptide N-**acetylgalactosaminyltransferase** is dependent on the amino acid sequences adjacent to serine and threonine residues
AU Wang, Yang; Agrwal, Neera; Eckhardt, Allen E.; Stevens, Robert D.; Hill, Robert L.
CS Med. Cent., Duke Univ., Durham, NC, 27710, USA
SO Journal of Biological Chemistry (1993), 268(31), 22979-83
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
CC 7-3 (Enzymes)
AB The acceptor substrate specificity of a pure polypeptide N-**acetylgalactosaminyltransferase** has been examd. with synthetic polypeptides with sequences identical, or similar to those found in porcine mucin or human **erythropoietin**. The sequences adjacent to either threonine or serine markedly influence the formation of GalNAc-O-Thr and GalNAc-O-Ser. Examn. of the mucin-like peptide VLGXXAV, where X is Thr, Ser, or Ala, shows only Thr-contg. peptides to be acceptors. The best substrate is formed when XX is TT. Peptides with XX as either AT or TA are less effective and those with XX as either ST or TS are much less effective acceptors. The amino acids adjacent to serine in the peptide formed by residues 121-131 in human **erythropoietin**, PPDAASAAPLR, also markedly influences the formation of GalNAc-O-Ser. Thus, PPDASSSAPLR and PPDVVSVVPLR are about 5- and 30-fold, resp., less active than the **erythropoietin** peptide. The peptide PPDGGSGGPLR is inactive. The shorter peptide DAASAAPL is also about 5-fold less active than the full-length peptide, but the peptide AASAA is inactive. These studies indicate that one transferase can form both GalNAc-O-Ser and GalNAc-O-Thr residues when the sequences adjacent to the **glycosylated** residue are of the proper kind. Thus, in contrast to earlier suggestions, there is no evidence that different transferases form GalNAc-O-Ser and GalNAc-O-Thr. Examn. of tissue homogenates from various tissues confirms this conclusion.

ST protein **acetylgalactosaminyltransferase** acceptor specifity sequence; threonine serine protein **acetylgalactosaminyltransferase** substrate

IT Protein sequences
(of protein **acetylgalactosaminyltransferase** model peptide substrates, reaction of peptide serine and threonine residues dependence on)

IT Michaelis constant
(of protein **acetylgalactosaminyltransferase**, of submaxillary gland, with model peptides)

IT Peptides, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(reaction of, with protein **acetylgalactosaminylatransferase** of submaxillary gland, kinetics of)

IT 56-45-1, Serine, biological studies 72-19-5, Threonine, biological studies
RL: BIOL (Biological study)
(of protein **acetylgalactosaminyltransferase** model peptide substrates, glycosylation dependence on sequence context of)

IT 143554-13-6 144574-84-5 149997-31-9 149997-32-0 149997-33-1 149997-34-2 149997-35-3 149997-36-4
RL: RCT (Reactant); RACT (Reactant or reagent)
(reaction of, with protein **acetylgalactosaminylatransferase** of submaxillary gland, kinetics of)

IT 9075-15-4, UDP N-**acetylgalactosamine** protein N-**acetylgalactosaminyltransferase**
RL: BIOL (Biological study)
(serine and threonine residues of model peptide substrates reaction with, of submaxillary gland, substrate amino acid sequence role in)

L60 ANSWER 27 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1993:441165 HCAPLUS
DN 119:41165
TI Structures of sialylated oligosaccharides of human erythropoietin expressed in recombinant BHK-21 cells
AU Nimtz, Manfred; Martin, Wolfgang; Wray, Victor; Kloeppel, Klaus Dieter; Augustin, Jan; Conradt, Harald S.
CS Dep. Cell Biol. Genet., GBF-Ges. Biotechnol. Forsch. mbH, Braunschweig, W-3300, Germany
SO European Journal of Biochemistry (1993), 213(1), 39-56
CODEN: EJBCAI; ISSN: 0014-2956
DT Journal
LA English
CC 2-2 (Mammalian Hormones)
AB The native structures of the Asn-linked oligosaccharides and the O-glycans at Ser126 of human **erythropoietin** expressed from recombinant BHK cells have been elucidated. Enzymically released N-glycans were studied by methylation analyses, fast-atom-bombardment mass spectrometry, and one- and two-dimensional 1H-NMR spectrometry at 600 MHz. Many (82.7%) were tetraantennary N-**acetyllactosamine**-type (22.8% with one, 3.6% with two, and 0.4% with three N-**acetyllactosamine** repeats) being tetrasialylated (41%), trisialylated (29.6%), and disialylated (12.2%). A few (9.7%; 4.1% 2,4-branched, 5.6% 2,6-branched) of the chains were triantennary (5.4% trisialyl, 4.3% disialyl) and 4.6% were of the disialyl diantennary type. Almost all of the innermost GlcNAc residues were .alpha.1-6 fucosylated and NeuAc was exclusively .alpha.2-3 linked to Gal.beta.1-4GlcNAc-R; 60% of the protein was O-**glycosylated** at Ser126; structures were monosialylated (70%) or disialylated (30%) forms of the Gal.beta.1-3GalNAc core type. **Glycosylation** patterns at individual Asn-Xaa-Thr/Ser sites were detd. by anal. high-pH anion-exchange chromatog. with pulsed amperometric detection. Only tetraantennary chains with 0-3 N-**acetyllactosamine** repeats were detected at Asn38 and Asn83, while almost all of the di- and triantennary

oligosaccharides were attached to Asn24. Batch anal. of different prepns. of recombinant **erythropoietin** revealed the high reproducibility of the prodn. procedure. Structures contg. terminal GalNAc-GlcNAc were detected in small amts. in a few batches.

ST sialylated oligosaccharide erythropoietin

IT Oligosaccharides
RL: BIOL (Biological study)
(**acetyllactosamine**-contg., branched, of human recombinant erythropoietin)

IT Oligosaccharides
RL: BIOL (Biological study)
(sialo-, of human recombinant erythropoietin)

IT 148046-46-2 148046-47-3 148046-48-4 148046-50-8 148046-52-0
148416-65-3 148416-66-4 148416-67-5 148416-68-6 148416-69-7
148416-70-0 148436-67-3 148436-68-4 148436-69-5
RL: BIOL (Biological study)
(of human recombinant erythropoietin)

IT 56-45-1, Serine, biological studies
RL: BIOL (Biological study)
(residue 126, of human recombinant erythropoietin, O-glycans of)

IT 70-47-3, Asparagine, biological studies
RL: BIOL (Biological study)
(residues 24 and 38 and 83, of human recombinant erythropoietin, oligosaccharides of)

IT 11096-26-7, Erythropoietin
RL: BIOL (Biological study)
(sialylated oligosaccharides of human recombinant)

L60 ANSWER 28 OF 36 HCAPLUS COPYRIGHT 2002 ACS

AN 1993:187209 HCAPLUS

DN 118:187209

TI Monosaccharide and oligosaccharide analysis of proteins transferred to polyvinylidene fluoride membranes after sodium dodecyl sulfate-polyacrylamide gel electrophoresis

AU Weitzhandler, Michael; Kadlecek, Douglas; Avdalovic, Nebojsa; Forte, John G.; Chow, Dar; Townsend, R. Reid

CS Dionex Corp., Sunnyvale, CA, 94088, USA

SO Journal of Biological Chemistry (1993), 268(7), 5121-30
CODEN: JBCHA3; ISSN: 0021-9258

DT Journal

LA English

CC 9-7 (Biochemical Methods)

AB An intermediate method was developed toward the complete carbohydrate anal. of proteins, which should be universally applicable to all proteins and independent of sample matrix. Using only Coomassie Blue-stained proteins that were electroblotted onto PVDF membranes, a strategy is reported for: (1) detg. unequivocally whether a protein is **glycosylated**; (2) obtaining a complete monosaccharide compn.; (3) oligosaccharide mapping which seps. most forms according to size, charge, and isomerism; and (4) sequentially releasing and analyzing specific classes of oligosaccharides with endoglycosidases. The method was applicable to a variety of well-characterized sol. glycoproteins and to the membrane-bound protein, the gastric H+,K+,-ATPase. The monosaccharide compn. of the H+,K+-ATPase revealed the absence of N-**acetylneuraminic** or N-glycolylneuraminic acids and a monosaccharide compn. which indicated O-linked sugar chains. Oligomannosidic/hybrid and biantennary oligosaccharides were sequentially released and analyzed from 1 electroblotted band of recombinant tissue plasminogen activator using endo-.beta.-N-**acetylglucosaminidase** H and endo-.beta.-N-**acetylglucosaminidase** F2, resp. Sialylated **polylactosamine** structures were identified and quantified by analyzing HPLC profiles of oligosaccharides first released by peptide-N4-(N-**acetyl**-.beta.-D-glucosaminyl)asparagine amidase

and then treated with endo-.beta.-galactosidase, using a single, stained band of recombinant **erythropoietin**. This recombinant **erythropoietin** contained 8 times more tetrasialylated oligosaccharides than previously reported (Sasaki, H. et al., 1987); 47% of released oligosaccharides were identified as **polylactosamine** structures.

ST glycoprotein sugar structure detn PAGE enzyme; gel electrophoresis saccharide detn glycoprotein; PVDF membrane glycoprotein immobilization analysis

IT Membrane, biological
(PVDF, glycoproteins immobilization on, for anal.)

IT Carbohydrates and Sugars, analysis
Monosaccharides
Oligosaccharides
RL: ANT (Analyte); ANST (Analytical study)
(anal. of, in glycoproteins by SDS-PAGE and electroblotting and enzyme treatment)

IT Molecular structure determination
(gel electrophoretic, of glycoproteins)

IT Glycoproteins, analysis
RL: ANST (Analytical study)
(sugars anal. in, by SDS-PAGE and electroblotting and enzyme treatment)

IT Chromatography, column and liquid
(anion-exchange, monosaccharide structure anal. by, in glycoproteins)

IT Electrophoresis and Ionophoresis
(gel, sugar anal. in glycoproteins by, electroblotting anal. after)

IT 37278-88-9
RL: ANST (Analytical study)
(H and F2 forms, sugar anal. in glycoproteins by)

IT 9000-83-3, ATPase
RL: ANST (Analytical study)
(hydrogen ion- and potassium-activated, sugar anal. of)

IT 24937-79-9, PVDF
RL: ANST (Analytical study)
(membrane, glycoproteins immobilization on, for carbohydrate anal.)

IT 9012-56-0, Amidase 52720-51-1, Endo-.beta.-galactosidase 52769-51-4, Endoglycosidase 83534-39-8
RL: ANST (Analytical study)
(sugar anal. in glycoproteins by)

IT 139639-23-9, Tissue plasminogen activator
RL: PROC (Process)
(sugar anal. of)

L60 ANSWER 29 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1992:647592 HCAPLUS
DN 117:247592
TI The influence of flanking sequence on the O-glycosylation of threonine in vitro
AU O'Connell, Brian C.; Hagen, Fred K.; Tabak, Lawrence A.
CS Sch. Med. Dent., Univ. Rochester, Rochester, NY, 14642, USA
SO Journal of Biological Chemistry (1992), 267(35), 25010-18
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
CC 7-3 (Enzymes)
AB To investigate the influence of flanking amino acid sequence on the O-**glycosylation** of a single threonine residue in vitro, a series of 52 related peptides were examd. The substrates were based upon a sequence from human von Willebrand factor which is known to be **glycosylated** in vivo (-6PHMAQVTVGPGL+5). Each residue of the parent peptide was substituted, in turn, with isoleucine, alanine, proline, glutamic acid, or arginine. Peptides were **glycosylated** using a UDP-GalNAc:polypeptide N-**acetylgalactosaminyltransferase**

purified 15,000-fold from bovine colostrum by chromatog. on DEAE-Sephacel, SP-Sephadex, Sephacryl S-300, Affi-Gel Blue, and 5-mercuri-UDP-GalNAc thiopropyl-Sepharose. Single amino acid changes in the sequences flanking the threonine could profoundly alter the **glycosylation** of the substrate peptides. Substitution of any amino acid tested at positions +3, -3, and -2 markedly decreased O-**glycosylation**, as did the presence of a charged residue at position -1. The substitution of amino acids at the other positions of the peptide substrate had little effect on the incorporation of GalNAc. Statistical anal. of sequences flanking known **glycosylated** threonine and serine residues suggests that they should be **glycosylated** with equal efficiency in the same sequence context (B. O'Connel et al., 1991). However, the bovine colostrum transferase failed to **glycosylate** a peptide derived from human **erythropoietin** which contains a serine that is **glycosylated** in vivo (-5PPDAASAAPLR+5). When a threonine was substituted for the serine in this peptide (-5PPDAATAAPLR+5), the substrate proved to be an excellent acceptor of GalNAc. These observations indicate that although flanking amino acid sequence is important for the O-**glycosylation** of specific hydroxyamino acids, discrete threonine- and serine-specific transferases may exist.

ST polypeptide **acetylgalactosaminyltransferase** threonine peptide substrate; UDP **acetylgalactosamine** polypeptide **acetylgalactosaminyltransferase** colostrum; glycosylation threonine peptide structure polypeptide **acetylgalactosaminyltransferase**

IT Colostrum
(UDP-**acetylgalactosamine**:polypeptide **acetylgalactosaminyltransferase** of, purifn and characterization of and peptide substrates for)

IT Michaelis constant
(of UDP-**acetylgalactosamine**: polypeptide **acetylgalactosaminyltransferase**, of colostrum)

IT Molecular structure-biological activity relationship
(polypeptide **acetylgalactosaminyltransferase**-substrate, of peptides)

IT Glycopeptides
RL: BIOL (Biological study)
(threonine-contg., glycosylation of threonine of, by polypeptide **acetylgalactosaminyltransferase** of colostrum, flanking sequence effects in relation to)

IT Proteins, specific or class
RL: BIOL (Biological study)
(threonine-rich, glycosylation of threonine of, by polypeptide **acetylgalactosaminyltransferase** of colostrum, flanking sequence effects in relation to)

IT 144574-32-3
RL: BIOL (Biological study)
(**acetylgalactosamine** incorporation in, by UDP-**acetylgalactosamine**:polypeptide **acetylgalactosaminyltransf erase**, peptide flanking amino acid sequence in relation to)

IT 72-19-5, Threonine, reactions
RL: RCT (Reactant); RACT (Reactant or reagent)
(glycosylation of, of peptides by polypeptide **acetylgalactosaminyltransferase**, peptide flanking sequence in relation to)

IT 1811-31-0, N-**Acetylgalactosamine**
RL: PROC (Process)
(incorporation of, in peptides by polypeptide **acetylgalactosaminyltransferase**, peptide flanking sequence in relation to)

IT 9075-15-4, UDP-N-**Acetylgalactosamine**:polypeptide N-**acetylgalactosaminyltransferase**
RL: BIOL (Biological study)
(peptides glycosylation by, of colostrum, peptide flanking sequence

effects in relation to)
IT 144574-33-4P 144574-34-5P 144574-35-6P 144574-36-7P 144574-37-8P
144574-38-9P 144574-39-0P 144574-40-3P 144574-41-4P 144574-42-5P
144574-43-6P 144574-44-7P 144574-45-8P 144574-46-9P 144574-47-0P
144574-48-1P 144574-49-2P 144574-50-5P 144574-51-6P 144574-52-7P
144574-53-8P 144574-54-9P 144574-55-0P 144574-56-1P 144574-57-2P
144574-58-3P 144574-59-4P 144574-60-7P 144574-61-8P 144574-62-9P
144574-63-0P 144574-64-1P 144574-65-2P 144574-66-3P 144574-67-4P
144574-68-5P 144574-69-6P 144574-70-9P 144574-71-0P 144574-72-1P
144574-73-2P 144574-74-3P 144574-75-4P 144574-76-5P 144574-77-6P
144574-78-7P 144574-79-8P 144574-80-1P 144574-81-2P 144574-82-3P
144574-83-4P 144597-16-0P
RL: SPN (Synthetic preparation); PREP (Preparation)
(prepn. and glycosylation by polypeptide **acetylgalactosaminyltransferase**, flanking sequence effects in relation to)
IT 144574-84-5 144574-85-6 144574-86-7 144574-87-8 144574-88-9
144597-17-1
RL: RCT (Reactant); RACT (Reactant or reagent)
(reaction of, with polypeptide **acetylgalactosaminyltransferase**, flanking sequence in relation to)

L60 ANSWER 30 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN **1991:651369** HCAPLUS
DN **115:251369**
TI Determination of the branch location of extra N-**acetyllactosamine** units in sialo N-linked tetraantennary oligosaccharides
AU Hokke, Cornelis H.; Kamerling, Johannis P.; Van Dedem, Gijs W. K.; Vliegenthart, Johannes F. G.
CS Bijovet Cent., Utrecht Univ., Utrecht, Neth.
SO FEBS Letters (1991), 286(1-2), 18-24
CODEN: FEBLAL; ISSN: 0014-5793
DT Journal
LA English
CC 9-5 (Biochemical Methods)
Section cross-reference(s): 6
AB An approach is presented for the detn. of the branch location of 1 or 2 extra N-**acetyllactosamine** units in sialo N-linked carbohydrate chains from glycoproteins. Tetraantennary oligosaccharides contg. extra N-**acetyllactosamine** units were digested with endo-.beta.-galactosidase, followed by treatment with N-acetyl-.beta.-glucosaminidase, yielding products which could be analyzed by 1H-NMR spectroscopy, thereby giving conclusive data about the location of the extra units in the intact structures.
ST **acetyllactosamine** structure branched sialooligosaccharide; NMR spectrometry glycoprotein structure
IT Molecular structure determination
(NMR spectrometric, **acetyllactosamine** branch location detn. in, of triantennary sialooligosaccharides from glycoproteins)
IT Sialoglycoproteins
RL: ANST (Analytical study)
(**acetyllactosamine**-contg., branch location detn. in oligosaccharides from, by NMR spectrometry)
IT Oligosaccharides
RL: ANST (Analytical study)
(sialo-, branched, **acetyllactosamine** branched location detn. in, by NMR spectrometry)
IT **11096-26-7, Erythropoietin**
RL: ANST (Analytical study)
(**acetyllactosamine** branch location detn. in oligosaccharides from, by NMR spectrometry)
IT 123430-80-8 137366-59-7 137366-60-0 137366-61-1
RL: ANST (Analytical study)

(**acetyllactosamine** branch location detn. in, by NMR spectrometry)

IT **32181-59-2**, N-**Acetyllactosamine**
RL: ANST (Analytical study)
(detn. of branched location of, in sialooligosaccharides by NMR spectrometry)

L60 ANSWER 31 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1991:551765 HCAPLUS
DN 115:151765
TI The importance of N- and O-linked oligosaccharides for the biosynthesis and in vitro and in vivo biologic activities of erythropoietin
AU Wasley, Louise C.; Timony, Gregg; Murtha, Patricia; Stoudemire, John; Dorner, Andrew J.; Caro, Jaime; Krieger, Monty; Kaufman, Randal J.
CS Genet. Inst., Cambridge, MA, 02140, USA
SO Blood (1991), 77(12), 2624-32
CODEN: BLOOAW; ISSN: 0006-4971
DT Journal
LA English
CC 2-10 (Mammalian Hormones)
AB **Erythropoietin** (**EPO**) plays a crit. role in stimulating the proliferation and differentiation of erythroid precursor cells. **EPO** is heavily **glycosylated** with three asparagine (N)-linked tetraantennary oligosaccharides that may contain N-**acetyl-lactosamine** repeats and a single serine (O)-linked oligosaccharide. **EPO** expressed in Chinese hamster ovary cells exhibits biol. properties and amino acid and carbohydrate compns. similar to those of natural urinary **EPO**. The importance of the complex N-linked and the O-linked carbohydrate was studied by expressing **EPO** in cells that are deficient in UDP-galactose/UDP-N-**acetylgalactosamine**-4-epimerase activity. In these cells, the ability to add galactose and N-**acetylgalactosamine** to glycoproteins can be controlled by the addn. of these sugars to the culture medium. The results demonstrate that a block in O-linked **glycosylation** and/or the ability to process N-linked carbohydrate to completion does not alter **EPO** secretion. **EPO** produced without O-linked carbohydrate exhibits normal in vitro and in vivo biol. activity and in vivo clearance. However, **EPO** produced with incompletely processed N-linked oligosaccharides exhibits normal in vitro activity but is at least 500-fold less effective in stimulating erythropoiesis in vivo. Studies on the survival of bioactive **EPO** remaining in the circulation demonstrated that **EPO** with incomplete N-linked oligosaccharides exhibits a 7-fold increased rate of clearance. However, this increased clearance may not fully account for the 500-fold loss of in vivo activity. These results suggest a potentially important unique requirement for appropriate complex N-linked oligosaccharides for the intrinsic biol. activity of **EPO** in vivo.
ST erythropoietin formation biol activity oligosaccharide
IT Oligosaccharides
RL: BIOL (Biological study)
(N- and O-linked, of erythropoietin, biol. activity and formation in relation to)
IT Erythropoiesis
(erythropoietin stimulation of, N-linked oligosaccharides role in)
IT 11096-26-7, Erythropoietin
RL: BIOL (Biological study)
(formation and biol. activity of, N- and O-linked oligosaccharides role in)

L60 ANSWER 32 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1991:2978 HCAPLUS
DN 114:2978

TI Structural characterization of glycoprotein carbohydrate chains by using digoxigenin-labeled lectins on blots
AU **Haselbeck, Anton**; Schickaneder, Edith; Von der Eltz, Herbert; Hoesel, Wolfgang
CS Biochem. Res. Cent., Boehringer Mannheim G.m.b.H., Tutzing, D-8132, Germany
SO Analytical Biochemistry (1990), 191(1), 25-30
CODEN: ANBCA2; ISSN: 0003-2697
DT Journal
LA English
CC 9-15 (Biochemical Methods)
Section cross-reference(s): 6, 33
AB The carbohydrate structures of blotted glycoproteins can be analyzed by probing them with lectins. Here a method is describe where lectins conjugated with digoxigenin are used in combination with an anti-digoxigenin antibody AP conjugate as a very sensitive detection system for this type of anal. The specificity of the lectins used, and the sensitivity of the detection system, provide valuable conclusions on the glycan structures. Only small amts. of glycoproteins are required for the anal. The binding specificity of a set of lectins is demonstrated with various glycoproteins of defined carbohydrate structure. The application of these labeled lectins in combination with specific glycosidases for the characterization of the carbohydrate chains of recombinant tissue plasminogen activator and **erythropoietin** is presented.
ST glycoprotein carbohydrate detn digoxigenin lectin electrophoresis
IT Agglutinins and Lectins
RL: ANST (Analytical study)
(carbohydrates of glycoproteins detected and evaluated by, following gel electrophoresis)
IT Glycoproteins, biological studies
RL: BIOL (Biological study)
(carbohydrates of, digoxigenin labeled lectins in anal. of)
IT Carbohydrates and Sugars, analysis
RL: ANT (Analyte); ANST (Analytical study)
(detn. of, of glycoproteins using digoxigenin-labeled lectins following gel electrophoresis)
IT Electrophoresis and Ionophoresis
(gel, glycoprotein carbohydrate anal. following, combined with digoxigenin-labeled lectins)
IT 1672-46-4D, Digoxigenin, conjugates with lectins
RL: ANST (Analytical study)
(carbohydrates of glycoproteins detected and evaluated by, following gel electrophoresis)
IT **11096-26-7, Erythropoietin**
RL: ANST (Analytical study)
(carbohydrates of, digoxigenin labeled lectins in anal. of)
IT 105913-11-9, Plasminogen activator
RL: ANST (Analytical study)
(tissue-type, carbohydrates of, digoxigenin-labeled lectins in anal. of)

L60 ANSWER 33 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1990:402848 HCAPLUS
DN 113:2848
TI Description and application of an immunological detection system for analyzing glycoproteins on blots
AU **Haselbeck, Anton**; Hoesel, Wolfgang
CS Biochem. Res. Cent., Boehringer Mannheim G.m.b.H., Tutzing, D-8132, Germany
SO Glycoconjugate Journal (1990), 7(1), 63-74
CODEN: GLJOEW; ISSN: 0282-0080
DT Journal

LA English
CC 9-10 (Biochemical Methods)
AB By introducing the steroid hapten digoxigenin specifically into sugars, a sensitive detection system for glycoproteins on blots has been developed. Sugars are oxidized to obtain aldehyde groups, which then react with digoxigenin-succinyl-.epsilon.-amido caproic acid hydrazide. A high-affinity antibody, conjugated to alk. phosphatase, is used for the detection of the incorporated digoxigenin. This system allows the detection of nanogram-amts. of glycoproteins on blots, and it's specificity allows a clear distinction of a glycoprotein from a non-glycoprotein. In combination with endo- and exoglycosidases, it is very useful for detg. the type of carbohydrate linkage in a glycoprotein, and by varying the oxidn. conditions, specific labeling of sialic acids and terminal galactoses can be achieved.
ST glycoprotein analysis blot immunochem
IT Fetuins
Glycoproteins, analysis
Orosomucoids
Sialic acids
RL: ANT (Analyte); ANST (Analytical study)
(detection of, on blots, immunol. detection system for)
IT Antibodies
RL: ANST (Analytical study)
(to digoxigenin, for immunol. detection of glycoproteins on blots)
IT Fetuins
RL: ANT (Analyte); ANST (Analytical study)
(asialo-, detection of, on blots, immunol. detection system for)
IT Electrophoresis and Ionophoresis
(gel, of glycoproteins, blotting after, immunol. detection system for)
IT 9046-67-7, Carboxypeptidase Y **11096-26-7, Erythropoietin**
RL: ANT (Analyte); ANST (Analytical study)
(detection of, on blots, immunol. detection system for)
IT 9001-67-6, Neuraminidase 9028-79-9, Galactose oxidase 9031-11-2, .beta.-Galactosidase 37278-88-9
RL: ANST (Analytical study)
(glycoproteins digestion with, for detection on blots using immunol. detection system)
IT 1672-46-4, Digoxigenin
RL: ANST (Analytical study)
(polyclonal antibodies to, for glycoproteins immunol. detection on blots)

L60 ANSWER 34 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1989:89389 HCAPLUS
DN 110:89389
TI Survival of recombinant **erythropoietin** in the circulation: the role of carbohydrates
AU Fukuda, Michiko N.; Sasaki, Hiroshi; Lopez, Lily; Fukuda, Minoru
CS Cancer Res. Cent., La Jolla Cancer Res. Found., La Jolla, CA, 92037, USA
SO Blood (1989), 73(1), 84-9
CODEN: BLOOAW; ISSN: 0006-4971
DT Journal
LA English
CC 2-10 (Mammalian Hormones)
AB To det. the role of carbohydrates in the stability of recombinant human **erythropoietin** in vivo, [125I]-labeled recombinant **erythropoietin** was i.v. infused into rats. The **erythropoietin** was slowly cleared from the blood with a half-life of .apprx.2 h. Asialoerythropoietin, which was produced by treatment of recombinant human **erythropoietin** with sialidase, was cleared rapidly from circulation within 10 min. Thus, the galactose-binding protein of hepatic cells is involved in the clearance of asialoerythropoietin. **Erythropoietin** also contains N-glycans

with a few N-**acetyllactosamine** repeats, which can be enriched by tomato lectin affinity chromatog. The lectin-bound fraction was cleared to a larger extent than was the unfractionated **erythropoietin**, whereas the component that did not bind the lectin was stable in the circulation. Authentic N-**acetyllactosamine** repeats (**polylactosaminoglycans**) prepd. from erythrocytes were similarly rapidly cleared from the circulation to the liver, and this clearance was inhibitable with asialo-.alpha.1 acid glycoprotein. Evidently, the sialic acid of the recombinant **erythropoietin** is necessary for this glycoprotein hormone to circulate stably and glycoproteins with >3 **lactosaminyl** repeat units may be cleared by the galactose-binding protein of hepatocytes.

ST **erythropoietin** metab liver carbohydrate moiety; blood **erythropoietin** stability carbohydrate moiety

IT Blood
(**erythropoietin** clearance from, carbohydrate moiety in relation to)

IT Liver, metabolism
(**erythropoietin** metab. by, carbohydrate moiety in relation to)

IT **Glycosidation**
Carbohydrates and Sugars, biological studies
Sialic acids
RL: BIOL (Biological study)
(of **erythropoietin**, liver metab. in relation to)

IT Oligosaccharides
RL: BIOL (Biological study)
(**acetyllactosamine**-contg., of **erythropoietin**, liver metab. in relation to)

IT Proteins, specific or class
RL: BIOL (Biological study)
(galactose-binding, of hepatocyte, recombinant human **erythropoietin** binding by)

IT **11096-26-7D, Erythropoietin**, asialo derivs.
RL: BIOL (Biological study)
(recombinant human, metab. of, by liver)

IT **11096-26-7, Erythropoietin**
RL: BIOL (Biological study)
(recombinant human, metab. of, by liver, carbohydrate moiety in relation to)

L60 ANSWER 35 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN 1988:605016 HCAPLUS
DN 109:205016
TI Site-specific **glycosylation** of human recombinant **erythropoietin**: analysis of glycopeptides or peptides at each **glycosylation** site by fast atom bombardment-mass spectrometry
AU Sasaki, Hiroshi; Ochi, Norimichi; Dell, Anne; Fukuda, Minoru
CS Cancer Res. Cent., La Jolla Cancer Res. Found., La Jolla, CA, 92037, USA
SO Biochemistry (1988), 27(23), 8618-26
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
CC 2-2 (Mammalian Hormones)
Section cross-reference(s): 6
AB To examine the extent to which protein structure influences **glycosylation**, the saccharide structures at each **glycosylation** site of human recombinant **erythropoietin**, i.e., Asn24, Asn38, Asn83, and Ser126, were analyzed by HPLC. Glycopeptides contg. different O-linked saccharides to the same peptide were sepd. Fast-atom bombardment mass spectrometry of the isolated glycopeptides combined with Edman degrdn. allowed elucidation of the compn. of the glycopeptides and the amino acid attachment site. The anal.

of glycopeptides and saccharides by fast-atom bombardment mass spectrometry and HPLC provided the following conclusions on N-glycans: (1) saccharides at Asn24 are heterogeneous and consist of biantennary, triantennary, and tetraantennary saccharides with or without N-**acetyllactosaminyl** repeats; (2) saccharides at Asn38 mainly consist of well-processed saccharides such as tetraantennary saccharides with or without N-**acetyllactosaminyl** repeats; (3) saccharides at Asn83, on the other hand, are homogeneous in the backbone structure and are composed mainly of tetraantennary saccharides without N-**acetyllactosaminyl** repeats. It was also noted that saccharides at Asn24 are much less sialylated than those at Asn38, although these 2 **glycosylation** sites are close to each other. Thus, the protein structure and, possibly, the carbohydrate chain at the neighboring site greatly influence **glycosylation** of a given **glycosylation** site.

ST **erythropoietin glycosylation** glycopeptide compn; protein **glycosylation** structure

IT Sialic acids
RL: BIOL (Biological study)
(of carbohydrate chains of human recombinant **erythropoietin**)

IT Carbohydrates and Sugars, biological studies
RL: BIOL (Biological study)
(of human recombinant **erythropoietin**, compn. of)

IT **Glycosidation**
(of human recombinant **erythropoietin**, sites for, compn. of)

IT **11096-26-7, Erythropoietin**
RL: BIOL (Biological study)
(human recombinant, **glycosylation** sites of, carbohydrate compn. of)

IT **32181-59-2**, N-**Acetyllactosamine**
RL: BIOL (Biological study)
(of carbohydrate chains, of glycosidation sites of human recombinant **erythropoietin**)

L60 ANSWER 36 OF 36 HCAPLUS COPYRIGHT 2002 ACS
AN **1988:2326** HCAPLUS
DN **108:2326**
TI Carbohydrate structure of **erythropoietin** expressed in Chinese hamster ovary cells by a human **erythropoietin** cDNA
AU Sasaki, Hiroshi; Bothner, Brian; Dell, Anne; Fukuda, Minoru
CS Cancer Res. Cent., La Jolla Cancer Res. Found., La Jolla, CA, 92037, USA
SO Journal of Biological Chemistry (1987), 262(25), 12059-76
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
CC 6-3 (General Biochemistry)
Section cross-reference(s): 2

AB Human **erythropoietins** were isolated from Chinese hamster ovary cells transfected with a human **erythropoietin** cDNA and from human urine. Carbohydrate chains attached to these proteins were isolated and fractionated by anion exchange HPLC and HPLC employing a Lichrosorb-NH2 column. The structures of fractionated saccharides were analyzed by fast-atom bombardment-mass spectrometry and methylation and anal. before and after treatment with specific exoglycosidases. Both **erythropoietins** contained 1 O-linked oligosaccharide/mol of the proteins, and its major component was NeuNAc.alpha.2.fwdarw.3Gal.beta.1.fwdarw.3(NeuNAc.alpha.2.fwdarw.6)GalNAcOH (where NeuNAc represents N-acetylneuraminic acid) in both proteins. The N-linked saccharides of recombinant **erythropoietin** consisted of biantennary (1.4% of the total saccharides), triantennary (10%), triantennary with 1 N-**acetyllactosaminyl** repeat (3.5%), tetraantennary (31.8%), and tetraantennary with 1 (32.1%), 2 (16.5%), or 3 (4.7%) N-**acetyllactosaminyl** repeats. All of these saccharides were

sialylated by 2.fwdarw.3-linkages. Tetraantennary with or without **polylactosaminyl** units were mainly present as disialosyl or trisialosyl forms, and these structures exhibited the following unique features. The .alpha.2.fwdarw.3-linked sialic acid and N-**acetyllactosaminoyl** repeats were selectively present in the side chains attached to C-6 and C-2 of 2,6-substituted .alpha.-mannose and C-4 of 2,4-substituted .alpha.-mannose. The carbohydrate moiety of urinary **erythropoietin** was indistinguishable from recombinant **erythropoietin** except for a slight difference in sialylation, which indicates that recombinant **erythropoietin** is valuable for biol. as well as clin. use.

ST **erythropoietin** carbohydrate structure urine recombinant

IT Urine
(**erythropoietin** of, carbohydrates of, structure of, of human)

IT Molecular structure, natural product
(of oligosaccharide, of recombinant and urinary **erythropoietin** of human)

IT Sialic acids
RL: BIOL (Biological study)
(of recombinant and urinary **erythropoietin**, of human)

IT Carbohydrates and Sugars, properties
Oligosaccharides
RL: PRP (Properties)
(structure of, of recombinant and urinary **erythropoietin** of human)

IT 75805-07-1 75805-13-9 75818-14-3 75847-86-8 75898-93-0
80968-33-8 80968-36-1 80968-37-2 80968-41-8 81024-62-6
111532-28-6 111532-29-7 111563-43-0 111589-50-5 111589-51-6
111589-52-7 111589-53-8 111618-14-5 111618-15-6 111618-16-7
RL: BIOL (Biological study)
(of **erythropoietin** recombinant of human)

IT **32181-59-2** 68366-20-1
RL: BIOL (Biological study)
(of recombinant and urinary **erythropoietin**, of human)

IT **11096-26-7, Erythropoietin**
RL: BIOL (Biological study)
(recombinant and urinary forms of, carbohydrates of, structure of, of human)

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YOU HAVE REQUESTED DATA FROM 13 ANSWERS - CONTINUE? Y/(N):y

L102 ANSWER 44 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:95748 BIOSIS
DN PREV200000095748
TI Protein-carbohydrate interactions in human lysozyme probed by combining site-directed mutagenesis and affinity labeling.
AU Muraki, Michiro (1); Harata, Kazuaki; Sugita, Naoki; Sato, Ken-ichi
CS (1) Biomolecules Department, National Institute of Bioscience and Human-Technology, 1-1 Higashi, Tsukuba, Ibaraki, 305-8566 Japan
SO Biochemistry, (Jan. 18, 2000) Vol. 39, No. 2, pp. 292-299.
ISSN: 0006-2960.
DT Article
LA English
SL English
AB The synergism between apolar and polar interactions in the carbohydrate recognition by human lysozyme (HL) was probed by site-directed mutagenesis and affinity labeling. The three-dimensional structures of the Tyr63fwdarwLeu mutant HL labeled with 2',3'-epoxypropyl beta-glycoside of N,N'-diacetylchitobiose (L63-HL/NAG-NAG-**EPO** complex) and the Asp102fwdarwGlu mutant HL labeled with the 2',3'-epoxypropyl

beta-glycoside of N-**acetyllactosamine** were revealed by X-ray diffraction at 2.23 and 1.96 ANG resolution, respectively. Compared to the wild-type HL labeled with the 2',3'-epoxypropyl beta-glycoside of N,N'-diacetylchitobiose, the N-acetylglucosamine residue at subsite B of the L63-HL/NAG-NAG-**EPO** complex markedly moved away from the 63rd residue, with substantial loss of hydrogen-bonding interactions. Evidently, the stacking interaction with the aromatic side chain of Tyr63 is essential in positioning the N-acetylglucosamine residue in the productive binding mode. On the other hand, the position of the galactose residue in subsite B of HL is almost unchanged by the mutation of Asp102 to Glu. Most hydrogen bonds, including the one between the carboxylate group of Glu102 and the axial 4-OH group of the galactose residue, were maintained by local movement of the backbone from residues 102-104. In both structures, the conformation of the disaccharide was conserved, reflecting an intrinsic conformational rigidity of the disaccharides. The structural analysis suggested that CH-pi interactions played an important role in the recognition of the carbohydrate residue at subsite B of HL.

CC Enzymes - Chemical and Physical *10806
Biochemical Studies - Proteins, Peptides and Amino Acids *10064
Biophysics - General Biophysical Techniques *10504
Biophysics - Molecular Properties and Macromolecules *10506
Enzymes - Methods *10804

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Methods and Techniques

IT Chemicals & Biochemicals
N,N'-diacetylchitobiose 2',3'-epoxypropyl beta-glycoside

IT Methods & Equipment
X-ray diffraction: X-ray analysis, analytical method; affinity labeling: analytical method, nucleic acid labeling; cation-exchange high performance liquid chromatography: analytical method, chromatographic techniques; crystallization: chemical modification, sample preparation method; site-directed mutagenesis: analytical method, mutagenesis, protein engineering

IT Miscellaneous Descriptors
protein-carbohydrate interactions

L102 ANSWER 45 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 2000:414216 BIOSIS
DN PREV200000414216
TI Asn-linked sugar chain structures of recombinant human thrombopoietin produced in Chinese hamster ovary cells.
AU Inoue, Noboru (1); Watanabe, Toshinori; Kutsukake, Toshiko; Saitoh, Hiroyuki; Tsumura, Haruhiko; Arai, Hirofumi; Takeuchi, Makoto
CS (1) Pharmaceutical Development Laboratory, KIRIN Brewery Co., Ltd., 100-1 Hagiwara-machi, Takasaki, Gunma, 370-0013 Japan
SO Glycoconjugate Journal, (November, 1999) Vol. 16, No. 11, pp. 707-718. print.
ISSN: 0282-0080.
DT Article
LA English
SL English
AB Human thrombopoietin (TPO) that regulates the numbers of megakaryocytes and platelets is a heavily N- and O-glycosylated glycoprotein hormone with partial homology to human **erythropoietin** (**EPO**). We prepared recombinant human TPO produced in Chinese hamster ovary (CHO) cells and analyzed the sugar chain structures quantitatively using 2-aminobenzamide labeling, sequential glycosidase digestion and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF/MS). We found bi-, tri- and tetraantennary complex-type sugar chains with one or two N-**acetyllactosamine** repeats, which are common to recombinant human **EPO** produced in CHO cells. On the other hand, there were triantennary sugar chains with

one or two N-**acetyllactosamine** repeats that were specific to the recombinant human TPO, and their distributions of branch structures were also different. These results suggested that proximal protein structure should determine the branch structure of Asn-linked sugar chains in addition to the glycosyltransferases subset.

CC Biochemical Studies - Proteins, Peptides and Amino Acids *10064
Cytology and Cytochemistry - Animal *02506
Pathology, General and Miscellaneous - Therapy *12512
Blood, Blood-Forming Organs and Body Fluids - Blood and Lymph Studies *15002
Blood, Blood-Forming Organs and Body Fluids - Blood Cell Studies *15004
Endocrine System - General *17002
Pharmacology - General *22002

BC Cricetidae 86310

IT Major Concepts
Endocrine System (Chemical Coordination and Homeostasis); Pharmacology

IT Parts, Structures, & Systems of Organisms
megakaryocytes: blood and lymphatics; platelet: blood and lymphatics

IT Chemicals & Biochemicals
asparagine; asparagine linked sugar chain structures; **erythropoietin** [**EPO**]; recombinant human thrombopoietin: glycoprotein hormone

IT Miscellaneous Descriptors
vaccine development

ORGN Super Taxa
Cricetidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia

ORGN Organism Name
CHO cell line (Cricetidae): Chinese hamster ovary cells

ORGN Organism Superterms
Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates

RN 70-47-3Q (ASPARAGINE)
3130-87-8Q (ASPARAGINE)
11096-26-7 (**ERYTHROPOIETIN**)

L102 ANSWER 46 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:86608 BIOSIS
DN PREV199900086608
TI Dual affinity labeling of the active site of human lysozyme with an N-**acetyllactosamine** derivative: First ligand assisted recognition of the second ligand.
AU Muraki, Michiro (1); Harata, Kazuaki; Sugita, Naoki; Sato, Ken-Ichi
CS (1) Biomolecules Dep., National Inst. Biosci. and Human-Technol., 1-1 Higashi, Tsukuba, Ibaraki 305-8566 Japan
SO Biochemistry, (Jan. 12, 1999) Vol. 38, No. 2, pp. 540-548.
ISSN: 0006-2960.
DT Article
LA English
AB Among the three kinds of the 2',3'-epoxypropyl beta-glycoside of disaccharides (GlcNAc-beta1,4-GlcNAc, Gal-beta1,4-GlcNAc, and Man-beta1,4-GlcNAc), the derivative of N-**acetyllactosamine** (Gal-beta1,4GlcNAc-**Epo**) caused the dual labeling of human lysozyme (HL) most efficiently. The labeled HL was crystallized and analyzed by X-ray diffraction methodology. The X-ray analysis located the two Galbeta1,4-GlcNAc-**Epo** moieties inside the catalytic cleft of HL. The attachment sites were the side-chain carboxylate groups of the catalytic residues Glu35 and Asp53 in HL. The first Gal-beta1,4-GlcNAc-**Epo** moiety occupied virtually the same position as observed in the HL labeled with single Gal-beta1,4-GlcNAc-**Epo** molecule. The second Gal-beta1,4-GlcNAc-**Epo** moiety was recognized via the carbohydrate-carbohydrate interaction with the first Gal-beta1,4-GlcNAc-**Epo** moiety in addition to the protein-carbohydrate interaction with the "right-side" catalytic cleft of HL through a number of hydrogen

bonds including water-mediated ones as well as many van der Waals contacts. The two N-acetylglucosamine residues stacked with each other, while the two rings of galactose residues approximately shared the same plane. The dual labeling with two Gal-beta1,4-GlcNAc-**Epo** molecules was supposed to have occurred sequentially, which was accompanied with the alteration to the pKa of Glu35 derived from the esterification of Asp53 in the first labeling. Both asymmetric carbons in the connection parts between HL and N-**acetyllactosamine** moieties showed the same stereoconfiguration derived from the reaction with (2R) stereoisomer concerning the epoxide group in the labeling reagent. The results demonstrated that the HL labeled with single Galbeta1,4-GlcNAc-**Epo** was functional as a novel N-**acetyllactosamine** -binding protein, and the second labeling was performed by way of the first-ligand assisted recognition of the second ligand.

CC Enzymes - Chemical and Physical *10806
Radiation - Radiation and Isotope Techniques *06504
Biochemical Studies - Proteins, Peptides and Amino Acids *10064
Biophysics - General Biophysical Techniques *10504

IT Major Concepts
Enzymology (Biochemistry and Molecular Biophysics); Methods and Techniques

IT Chemicals & Biochemicals
human lysozyme [EC 3.2.1.17]: analysis, crystallization, labeling; N-**acetyllactosamine** derivative: label

IT Methods & Equipment
lysozyme crystallization: crystallization techniques, sample preparation method; reversed phase column chromatography: separation method, size exclusion chromatography; MALDI-TOF/MS [matrix-assisted laser desorption ionization/time of flight mass spectrometry]: analytical method, mass spectrometry: CB; X-ray diffraction: X-ray analysis, analytical method

RN 9001-63-2 (LYSOZYME)
32181-59-2D (N-**ACETYLLACTOSAMINE**)
9001-63-2 (EC 3.2.1.17)

L102 ANSWER 47 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1999:317189 BIOSIS
DN PREV199900317189
TI Establishment of a method for mapping of N-linked oligosaccharides and its use to analyze industrially produced recombinant **erythropoietin**.
AU Kanazawa, Koki (1); Ashida, Kyoko; Itoh, Masao; Nagai, Hiroshi; Sasaki, Hiroshi; Fukuda, Minoru
CS (1) Pharmaceutical Production Division, Chugai Pharmaceutical Co., Ltd., 5-5-1 Ukima, Kita-ku, Tokyo, 115-8543 Japan
SO Biological & Pharmaceutical Bulletin, (April, 1999) Vol. 22, No. 4, pp. 339-346.
ISSN: 0918-6158.
DT Article
LA English
SL English
AB A rapid and convenient method for N-linked oligosaccharide structure analysis was developed and applied to the quality examination of commercially manufactured recombinant human **erythropoietin** (rEPO). Oligosaccharides released from rEPO expressed in Chinese hamster ovary cells were labeled with 2-aminobenzamide, and analyzed by a combination of diethylaminoethyl (DEAE) column HPLC and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF-MS). This newly developed method allowed us to analyze sialylated oligosaccharides directly. The results showed that these oligosaccharides are composed of neutral-mono-di-tri-tetrasialyl oligosaccharides in a ratio of <1 : 2 : 13 : 34 : 51%, and that the core structure was a mixture of complex type bi-, tri- and tetra-antennary forms with one to three **polylactosaminyl** chains. Further

analysis showed clearly that the N-linked oligosaccharide structure of rEPO has not changed throughout the past ten years of manufacturing by our company. The analysis was carried out using a small quantity (1 nmol) of rEPO, demonstrating the efficacy of the newly developed method. Further, the N-linked oligosaccharides of rEPO manufactured by our company almost coincided with those of the **erythropoietin** European Pharmacopoeia Biological Reference Preparation (Eur.Ph.BRP), a standard product.

CC Biochemical Methods - General *10050
Biochemical Studies - General *10060
Biophysics - General Biophysical Studies *10502

BC Cricetidae 86310

IT Major Concepts
Biochemistry and Molecular Biophysics; Methods and Techniques

IT Chemicals & Biochemicals
erythropoietin: industrially produced, recombinant; sialyated oligosaccharides; 2-aminobenzamide

IT Methods & Equipment
diethylaminoethyl column high performance liquid chromatography: analytical method; matrix assisted laser desorption ionization time of flight mass spectroscopy: analytical method

ORGN Super Taxa
Cricetidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia

ORGN Organism Name
CHO cell line (Cricetidae)

ORGN Organism Superterms
Animals; Chordates; Mammals; Nonhuman Mammals; Nonhuman Vertebrates; Rodents; Vertebrates

RN **11096-26-7** (**ERYTHROPOIETIN**)
88-68-6 (2-AMINOBENZAMIDE)

L102 ANSWER 48 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1996:536487 BIOSIS
DN PREV199699258843
TI Origin of carbohydrate recognition specificity of human lysozyme revealed by affinity labeling.
AU Muraki, Michiro (1); Harata, Kazuaki; Sugita, Naoki; Sato, Ken-Ichi
CS (1) Biomolecules Dep., Natl. Inst. Bioscience Hum. Technol., Tsukuba, Ibaraki 305 Japan
SO Biochemistry, (1996) Vol. 35, No. 42, pp. 13562-13567.
ISSN: 0006-2960.
DT Article
LA English
AB In order to reveal the origin of carbohydrate recognition specificity of human lysozyme by clarifying the difference in the binding mode of ligands in the active site, the inactivation of human lysozyme by 2',3'-epoxypropyl beta-glycoside derivatives of the disaccharides, N,N'-diacetylchitobiose (GlcNAc-beta-(1 fwdarw 4)-GlcNAc) and N-**acetyllactosamine** (Gal-beta-(1 fwdarw 4)-GlcNAc), was investigated and the three-dimensional structures of the affinity-labeled enzymes were determined by X-ray crystallography at 1.7 ANG resolution. Under the conditions comprising 2.0 times 10-3 M labeling reagent and 1.0 times 10-5 M human lysozyme at pH 5.4, 37 degree C, the reaction time required to reduce the lytic activity against Micrococcus luteus cells to 50% of its initial activity was lengthened by 3.7 times through the substitution of the nonreducing end sugar residue, GlcNAc to Gal. The refined structure of human lysozyme labeled by 2',3'-epoxypropyl beta-glycoside derivatives of N,N-diacetylchitobiose (HL/NAG-NAG-**EPO** complex) indicated that the interaction mode of the N,N'-diacetylchitoblose moiety in substites B and C in this study was essentially the same as in the case of the complex of human lysozyme with the free ligand. On the other hand, the hydrogen-bonding pattern and the stacking interaction at subsite B were remarkably different between the HL/NAG-NAG-**EPO** complex and

human lysozyme labeled by the 2',3'-epoxypropyl beta-glycoside of N-**acetyllactosamine** (HL/GAL-NAG-**EPO** complex). The reduced number of possible hydrogen bonds as well as the less favorable stacking between the side chain of Tyr63 in human lysozyme and the galactose residue in the HL/GAL-NAG-**EPO** complex reasonably explained the less efficient ability of the 2',3'-epoxypropyl beta-glycoside of N-**acetyllactosamine** as compared to that of N,N'-diacetylchitobiose as an affinity labeling reagent toward human lysozyme.

CC Biochemical Studies - Proteins, Peptides and Amino Acids *10064
Biochemical Studies - Carbohydrates *10068
Biophysics - Molecular Properties and Macromolecules *10506
Enzymes - Chemical and Physical *10806
Enzymes - Physiological Studies *10808
Morphology and Cytology of Bacteria *30500

BC Micrococcaceae *07702

IT Major Concepts
Biochemistry and Molecular Biophysics; Cell Biology; Enzymology (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
LYSOZYME; N-**ACETYLLACTOSAMINE**

IT Miscellaneous Descriptors
AFFINITY LABELING; ANALYTICAL METHOD; CARBOHYDRATE RECOGNITION SPECIFICITY; ENZYMOLOGY; HUMAN LYSOZYME; HUMAN LYSOZYME BINDING; LYSIS; LYTIC ACTIVITY; N-**ACETYLLACTOSAMINE**; N,N'-DIACETYLCHITOBIOSE; THREE-DIMENSIONAL STRUCTURE; 2',3'-EPOXYPROPYL BETA-GLYCOSIDE

ORGN Super Taxa
Micrococcaceae: Eubacteria, Bacteria

ORGN Organism Name
Micrococcus luteus (Micrococcaceae)

ORGN Organism Superterms
bacteria; eubacteria; microorganisms

RN 9001-63-2 (LYSOZYME)
32181-59-2 (N-**ACETYLLACTOSAMINE**)

L102 ANSWER 49 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1995:391061 BIOSIS
DN PREV199598405361
TI Kinetic analysis of a recombinant UDP-N-acetyl-D-**galactosamine**: Polypeptide N-**acetylgalactosaminyltransferase**.
AU Wragg, Stephanie; Hagen, Fred K.; Tabak, Lawrence A. (1)
CS (1) Dep. Dent. Res. Biochem., Sch. Med. Dent., Univ. Rochester, 601 Elmwood Ave., Box 611, Rochester, NY 14642 USA
SO Journal of Biological Chemistry, (1995) Vol. 270, No. 28, pp. 16947-16954. ISSN: 0021-9258.
DT Article
LA English
AB A mammalian expression vector was designed to express a secreted soluble form of the UDP-GalNAC: polypeptide N-**acetylgalactosaminyltransferase** (polypeptide GalNAc transferase) with a metal binding site (HHWHHH) at the NH-2 terminus. The recombinant enzyme was purified to homogeneity from COS-7 cell media by sequential chromatography on columns of NiCl-2-chelating Sepharose, Affi-Gel blue, and Sephacryl S-100. Kinetic parameters of recombinant and native polypeptide GalNAc transferase were comparable for the donor UDP-GalNAc and for the peptide acceptors AcTPPP, **EPO**-T (PPDAATAAPLR), and HVF (PHMAQVTVGPGL). Initial velocity and product inhibition studies were carried out with purified recombinant polypeptide GalNAc transferase and the substrates UDP-GalNAc and peptide **EPO**-T. Initial velocity data was consistent with a sequential type mechanism in which binding of both substrates precedes product release. Product inhibition analysis using UDP showed competitive inhibition against UDP-GalNAc and a noncompetitive inhibition against peptide **EPO**-T. The dead end peptide analogue **EPO**-G (PPDAAGAAPLR) was a noncompetitive inhibitor of UDP-GalNAc and a competitive inhibitor

of peptide **EPO**-T. Collectively, the results suggest that the most probable kinetic mechanism for the enzyme is one in which both substrates must bind in a random order prior to catalysis. Interestingly, the K-m for **EPO**-T is similar to the K-i for **EPO**-G, suggesting that peptide interaction with the polypeptide GalNAc transferase does not require a hydroxyamino acid.

CC Cytology and Cytochemistry - Animal 02506
Genetics and Cytogenetics - Animal 03506
Biochemical Studies - Proteins, Peptides and Amino Acids 10064
Biochemical Studies - Carbohydrates *10068
Biophysics - Molecular Properties and Macromolecules *10506
Enzymes - Chemical and Physical *10806

BC Osteichthyes *85206

IT Major Concepts
Biochemistry and Molecular Biophysics; Enzymology (Biochemistry and Molecular Biophysics)

IT Chemicals & Biochemicals
UDP-N-ACETYL-D-**GALACTOSAMINE**; N-**ACETYLGALACTOSAMINYLTRANSFERASE**

IT Miscellaneous Descriptors
ENZYME KINETICS; PURIFICATION METHOD; SEQUENTIAL CHROMATOGRAPHY

ORGN Super Taxa
Cercopithecidae: Primates, Mammalia, Vertebrata, Chordata, Animalia; Osteichthyes: Pisces, Vertebrata, Chordata, Animalia

ORGN Organism Name
COS-7 (Cercopithecidae): cell line; Osteichthyes (Osteichthyes)

ORGN Organism Superterms
animals; chordates; fish; mammals; nonhuman mammals; nonhuman primates; nonhuman vertebrates; primates; vertebrates

RN 7277-98-7 (UDP-N-ACETYL-D-**GALACTOSAMINE**)
9054-44-8 (N-**ACETYLGALACTOSAMINYLTRANSFERASE**)

L102 ANSWER 50 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

AN 1995:371009 BIOSIS

DN PREV199598385309

TI Antifreeze glycopeptides of the high-Antarctic silverfish Pleurogramma antarcticum (Notothenioidei.

AU Woehrmann, Andreas P. A.

CS Inst. Polaroekol., Univ. Kiel, Wischhofstrasse 1-3, Gebaede 12, D-24148 Kiel Germany

SO Comparative Biochemistry and Physiology C Pharmacology Toxicology & Endocrinology, (1995) Vol. 111, No. 1, pp. 121-129.
ISSN: 0742-8413.

DT Article

LA English

AB Antifreeze glycopeptides (AFGP) have been isolated from the fully pelagic high-Antarctic silverfish Pleuragramma antarcticum of the suborder Notothenioidei (Perciformes). The fishes were caught during the PRV Polarstern expedition **EPOS** III (Jan-Mar, 1989) in the eastern and southeastern Weddell Sea. Glycoconjugate and amino acid analysis of antifreeze glycopeptides (AFGP) indicate that the glycopeptide structure is identical to the polymers of H-2N(Ala-Ala(beta-galactosyl(1 fwdarw 3)alpha-N-**acetylgalactosamine**)Thr)-nAla-Ala-COOH of previously studied Antarctic notothenioids. The content of AFGPs in P. antarcticum is lower than in other notothenioid fish from the same region. Antifreeze activity shows a maximal hysteresis of 1.19 degree C at a concentration of 20 mg/ml AFGP. A linear increase in activity of the antifreeze glycopeptides could be demonstrated concomitant with a decreasing ice content. The freezing point of blood serum is -1.9 degree C.

CC Ecology; Environmental Biology - Animal *07508
Ecology; Environmental Biology - Oceanography *07512
Biochemical Studies - Proteins, Peptides and Amino Acids 10064
Biophysics - Molecular Properties and Macromolecules *10506

External Effects - Temperature as a Primary Variable - Cold *10616
Metabolism - Carbohydrates *13004
Metabolism - Proteins, Peptides and Amino Acids *13012
Blood, Blood-Forming Organs and Body Fluids - Blood and Lymph Studies *15002
Temperature: Its Measurement, Effects and Regulation - Cryobiology *23004
Temperature: Its Measurement, Effects and Regulation - Thermoadaptation *23010
BC Osteichthyes *85206
IT Major Concepts
Biochemistry and Molecular Biophysics; Blood and Lymphatics (Transport and Circulation); Ecology (Environmental Sciences); Marine Ecology (Ecology, Environmental Sciences); Metabolism; Physiology
IT Miscellaneous Descriptors
DIFFERENTIAL SCANNING CALORIMETRY; THERMAL HYSTERESIS; WEDDELL SEA
GT South Atlantic Ocean (Atlantic Ocean)
ORGN Super Taxa
Osteichthyes: Pisces, Vertebrata, Chordata, Animalia
ORGN Organism Name
Pleurogramma antarcticum (Osteichthyes)
ORGN Organism Superterms
animals; chordates; fish; nonhuman vertebrates; vertebrates

L102 ANSWER 51 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1994:298658 BIOSIS
DN PREV199497311658
TI Structure determination of the intact major sialylated oligosaccharide chains of recombinant human **erythropoietin** expressed in Chinese hamster ovary cells.
AU Watson, Eric (1); Bhide, Anjali (1); Van Halbeek, Herman
CS (1) Amgen Inc., Amgen Cent., Thousand Oaks, 1840 DeHavilland Dr., CA 91320-1789 USA
SO Glycobiology, (1994) Vol. 4, No. 2, pp. 227-237.
ISSN: 0959-6658.
DT Article
LA English
AB Recombinant human **erythropoietin** (rHuEPO) is used abundantly in the clinic to stimulate red blood cell growth in anaemic patients. The efficacy of the drug depends strongly on the extent of sialylation of its carbohydrate moiety. Prompted by conflicting literature reports on the issue, we reinvestigated the structures of the intact sialylated carbohydrate chains of rHuEPO expressed in Chinese hamster ovary (CHO) cells. The asparagine-linked oligosaccharides were released from rHuEPO with N-glycanase and fractionated by anion-exchange chromatography. The O-linked oligosaccharides were released under alkaline borohydride conditions. The primary structures of the major sialylated N- and O-type oligosaccharides were identified by 500-MHz 1H-NMR spectroscopy, supported by data from composition analysis, methylation analysis, low- and high-pH anion-exchange chromatography, and fast atom bombardment-mass spectrometry. The most abundant N-linked oligosaccharides in CHO cell-derived rHuEPO were found to be di-antennary, 2,4-branched tri-antennary, 2,6-branched tri-antennary and tetra-antennary chains (in the ratio of 7:6:5:82), with the latter containing between zero and three repeating N-**acetyllactosamine** units, in well-defined branches. The major (gt 95%) di-, tri- and tetra-antennary structures are fully sialylated, i.e. they have two, three and four sialic acid residues, respectively, linked exclusively alpha(2 fwdarw 3) to galactose residues. The majority (gt 95%) of N-linked structures contain alpha(1 fwdarw 6)-linked fucose at the proximal GlcNAc residue. The O-type mono- and disialyl oligosaccharides were characterized as a linear tri- and a branched tetra-saccharide, respectively.
CC Cytology and Cytochemistry - Animal *02506
Biochemical Studies - Proteins, Peptides and Amino Acids *10064

Biochemical Studies - Carbohydrates *10068
Biophysics - Molecular Properties and Macromolecules *10506
Blood, Blood-Forming Organs and Body Fluids - Blood and Lymph Studies *15002
Endocrine System - General *17002
BC Hominidae *86215
IT Major Concepts
Biochemistry and Molecular Biophysics; Blood and Lymphatics (Transport and Circulation); Cell Biology; Endocrine System (Chemical Coordination and Homeostasis)
IT Chemicals & Biochemicals
ERYTHROPOIETIN
IT Miscellaneous Descriptors
FAST ATOM BOMBARDMENT MASS SPECTROMETRY; NMR
ORGN Super Taxa
Hominidae: Primates, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name
Hominidae (Hominidae)
ORGN Organism Superterms
animals; chordates; humans; mammals; primates; vertebrates
RN **11096-26-7 (ERYTHROPOIETIN)**

L102 ANSWER 52 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1993:54751 BIOSIS
DN PREV199395031053
TI Quantitative mapping of the N-linked sialyloligosaccharides of recombinant **erythropoietin**: Combination of direct high-performance anion-exchange chromatography and 2-aminopyridine derivatization.
AU Rice, Kevin G.; Takahashi, Noriko; Namiki, Yoshihiro; Tran, An D.; Lisi, Peter J.; Lee, Yuan C. (1)
CS (1) Dep. Biol., Johns Hopkins Univ., Baltimore, Md. 21218
SO Analytical Biochemistry, (1992) Vol. 206, No. 2, pp. 278-287. ISSN: 0003-2697.
DT Article
LA English
AB A rapid quantitative analysis of the sialylated N-linked oligosaccharides of recombinant **erythropoietin** (**EPO**) expressed in Chinese hamster ovary (CHO) cells has been developed. The procedure utilizes a glycoamidase (glycopeptidase F) to release all of the N-linked oligosaccharides from the native glycoprotein, followed by direct chromatographic analysis using high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection. The eight sialyloligosaccharides isolated from HPAEC were characterized by derivatizing with 2-aminopyridine followed by two-dimensional HPLC mapping of the pyridylaminated asialooligosaccharides (Tomiya et al., 1988, Anal. Biochem. 171, 73-90). Seven kinds of complex-type asialooligosaccharides were identified ranging from a biantennary structure to N-**acetyllactosamine**-extended tetraantennary structure. Approximately 3% of the terminal galactose residue of the oligosaccharides released from **EPO** were not sialylated whereas 97% contained an alpha(2 fwdarw 3)-linked sialic acid. Quantitative oligosaccharide mapping of four different lots of **EPO** from CHO cells was performed to quantify the molar balance and distribution of the N-linked oligosaccharides. The sialyloligosaccharides were distributed with approximately 5% disialylated (single type), 20% trisialylated (six types), and 75% tetrasialyated (four types) oligosaccharides with an average molar recovery of 85% starting from 750 pmol of **EPO**.
CC Cytology and Cytochemistry - Animal *02506
Biochemical Methods - Carbohydrates *10058
Biochemical Studies - Proteins, Peptides and Amino Acids 10064
Biochemical Studies - Carbohydrates 10068
Biophysics - General Biophysical Techniques *10504
Biophysics - Molecular Properties and Macromolecules *10506

Endocrine System - General *17002
Pharmacology - Blood and Hematopoietic Agents *22008
BC Cricetidae *86310
IT Major Concepts
Biochemistry and Molecular Biophysics; Cell Biology; Endocrine System (Chemical Coordination and Homeostasis); Methods and Techniques; Pharmacology
IT Chemicals & Biochemicals
ERYTHROPOIETIN; 2-AMINOPYRIDINE; N-**ACETYLGALACTOSAMINE**
IT Miscellaneous Descriptors
ALPHA-2 3=LINKED SIALIC ACID; ANALYTICAL METHOD; N=**ACETYLGALACTOSAMINE**
ORGN Super Taxa
Cricetidae: Rodentia, Mammalia, Vertebrata, Chordata, Animalia
ORGN Organism Name
hamster (Cricetidae); CHO (Cricetidae): cell line
ORGN Organism Superterms
animals; chordates; mammals; nonhuman mammals; nonhuman vertebrates; rodents; vertebrates
RN **11096-26-7** (**ERYTHROPOIETIN**)
504-29-0 (2-AMINOPYRIDINE)
1811-31-0Q (N-**ACETYLGALACTOSAMINE**)
31022-50-1Q (N-**ACETYLGALACTOSAMINE**)

L102 ANSWER 53 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1988:218615 BIOSIS
DN BA85:107850
TI COMPARATIVE STUDY OF THE ASPARAGINE-LINKED SUGAR CHAINS OF HUMAN **ERYTHROPOIETINS** PURIFIED FROM URINE AND THE CULTURE MEDIUM OF RECOMBINANT CHINESE HAMSTER OVARY CELLS.
AU TAKEUCHI M; TAKASAKI S; MIYAZAKI H; KATO T; HOSHI S; KOCHIBE N; KOBATA A
CS DEP. BIOCHEM., INST. MED. SCI., UNIV. TOKYO, MINATO-KU, TOKYO 108, JPN.
SO J BIOL CHEM, (1988) 263 (8), 3657-3663.
CODEN: JBCHA3. ISSN: 0021-9258.
FS BA; OLD
LA English
AB The asparagine-linked sugar chains of human **erythropoietin** produced by recombinant Chinese hamster ovary cells and naturally occurring human urinary **erythropoietin** were liberated by hydrazinolysis and fractionated by paper electrophoresis, lectin affinity chromatography, and Bio-Gel P-4 column chromatography. Both **erythropoietins** had three asparagine-linked sugar chains in one molecule, all of which were acidic complex type. Structural analysis of them revealed that the sugar chains from both **erythropoietins** are quite similar except for sialyl linkage. All sugar chains of **erythropoietin** produced by recombinant Chinese hamster ovary cells contain only the NeuAc.alpha.2 .fwdarw. 3Gal linkage, while those of human urinary **erythropoietin** contain the NeuAc.alpha.2 .fwdarw. 6Gal linkage together with the NeuAc.alpha.2 .fwdarw. 3Gal linkage. The major sugar chains were of fucosylated tetraantennary complex type with and without N-**acetyllactosamine** repeating units in their outer chain moieties in common, and small amounts of 2,4- and 2,6-branched triantennary and biantennary sugar chains were detected. This paper proved, for the first time, that recombinant technique can produce glycoprotein hormone whose carbohydrate structures are common to the major sugar chains of the native one.
CC Cytology and Cytochemistry - Animal *02506
Cytology and Cytochemistry - Human *02508
Comparative Biochemistry, General 10010
Biochemical Methods - Proteins, Peptides and Amino Acids 10054
Biochemical Methods - Carbohydrates 10058
Biochemical Studies - Proteins, Peptides and Amino Acids *10064

Biochemical Studies - Carbohydrates *10068
Biophysics - General Biophysical Techniques 10504
Biophysics - Molecular Properties and Macromolecules *10506
Blood, Blood-Forming Organs and Body Fluids - Lymphatic Tissue and Reticuloendothelial System *15008
Endocrine System - General *17002
BC Hominidae 86215
Cricetidae 86310
RN 11096-26-7D (**ERYTHROPOIETINS**)
70-47-3Q, 7006-34-0Q (ASPARAGINE)

L102 ANSWER 54 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1985:378558 BIOSIS
DN BA80:48550
TI THE ROLE OF CARBOHYDRATE IN **ERYTHROPOIETIN** ACTION.
AU DORDAL M S; WANG F F; GOLDWASSER E
CS DEP. OF BIOCHEMISTRY AND MOLECULAR BIOLOGY, THE UNIVERSITY OF CHICAGO, 920 E. 58TH ST., CHICAGO, ILL. 60637.
SO ENDOCRINOLOGY, (1985) 116 (6), 2293-2299.
CODEN: ENDOAO. ISSN: 0013-7227.
FS BA; OLD
LA English
AB The carbohydrate composition of human **erythropoietin** (**epo**) was determined by micro-GLC. Enzymic removal of most of the sugars results in aggregation of glycosidase-treated **epo**, loss of biological activity when assayed in mice, and retention of activity when assayed in marrow cell cultures or by RIA [radioimmunoassay]. Endoglycosidase F causes the removal of most of the carbohydrates, indicating that the oligosaccharides are asparagine linked. The lack of O-linked sugar is confirmed by the absence of N-**acetylgalactosamine**. Apparently, the oligosaccharide portion of **epo**, although required for action in vivo, is not required for interaction with the target cells of the bloodforming system.
CC Cytology and Cytochemistry - Animal *02506
Cytology and Cytochemistry - Human *02508
Biochemical Studies - Proteins, Peptides and Amino Acids 10064
Biochemical Studies - Carbohydrates *10068
Enzymes - Methods 10804
Blood, Blood-Forming Organs and Body Fluids - Blood and Lymph Studies *15002
Endocrine System - General *17002
Tissue Culture, Apparatus, Methods and Media 32500
BC Hominidae 86215
Muridae 86375
IT Miscellaneous Descriptors
MOUSE HUMAN ENDOGLYCOSIDASE F GLYCOSIDASE
RN 9032-92-2 (GLYCOSIDASE)
11096-26-7 (**ERYTHROPOIETIN**)
52769-51-4 (ENDOGLYCOSIDASE)

L102 ANSWER 55 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1982:155183 BIOSIS
DN BA73:15167
TI METABOLIC STUDIES ON **ERYTHROPOIETIN** 2. THE ROLE OF LIVER AND KIDNEY IN THE METABOLISM OF **ERYTHROPOIETIN**.
AU DINKELAAR R B; ENGELS E Y; HART A A M; SCHOEMAKER L P; BOSCH E; CHAMULEAU R A F M
CS DIAKONESSENHUIS "REFAJA", P.O. BOX 444, 3300 AK DORDRECHT, NETH.
SO EXP HEMATOL (LAWRENCE), (1981) 9 (7), 796-803.
CODEN: EXHMA6. ISSN: 0301-472X.
FS BA; OLD
LA English
AB Half plasma disappearance time (HDT) of endogenous **erythropoietin**

(Ep) was measured in single rats, using an in vitro assay system for Ep. In rats treated with the hepatotoxic agent d-**Galactosamine**-HCl (GalN), a small but significant elevation of HDT was found as compared with control rats (164 and 105 min, respectively). In bilaterally nephrectomized rats mean HDT was significantly elevated: 266 min. Combination of nephrectomy and GalN treatment did not result in a significant further elongation of HDT (.hivin.x = 301 min). In experiments using isolated liver perfusion, rat livers (with and without GalN treatment) were unable to change perfusate Ep titer during 4 h of perfusion. Hepatic degradation of Ep in rats is only minimal. The kidney, however, is important in the catabolism of Ep.

CC Biochemical Studies - Proteins, Peptides and Amino Acids 10064
Biochemical Studies - Carbohydrates 10068
Anatomy and Histology, General and Comparative - Experimental Anatomy 11104
Metabolism - Nucleic Acids, Purines and Pyrimidines *13014
Digestive System - General; Methods 14001
Digestive System - Physiology and Biochemistry *14004
Cardiovascular System - General; Methods 14501
Blood, Blood-Forming Organs and Body Fluids - Blood and Lymph Studies *15002
Urinary System and External Secretions - General; Methods 15501
Urinary System and External Secretions - Physiology and Biochemistry *15504
Endocrine System - General *17002
Toxicology - General; Methods and Experimental 22501

BC Muridae 86375

IT Miscellaneous Descriptors
RAT DEXTRO **GALACTOSAMINE** HEPATO TOXIC AGENT NEPHRECTOMY LIVER PERFUSION

RN **11096-26-7** (**ERYTHROPOIETIN**)

L102 ANSWER 56 OF 56 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1979:173557 BIOSIS
DN BA67:53557
TI USE OF IMMOBILIZED LECTINS AND OTHER LIGANDS FOR THE PARTIAL PURIFICATION OF **ERYTHROPOIETIN**.
AU SPIVAK J L; SMALL D; SHAPER J H; HOLLENBERG M D
CS CLAYTON LAB., TRAYLOR 924, 720 RUTLAND AVE., BALTIMORE, MD. 21205, USA.
SO BLOOD, (1978) 52 (6), 1178-1188.
CODEN: BLOOAW. ISSN: 0006-4971.
FS BA; OLD
LA English
AB The ability of a variety of affinity adsorbents to isolate [human] **erythropoietin** (Ep) from contaminating proteins in crude preparations of the hormone was examined. Of 13 lectin-agarose derivatives, 6 bound Ep but only 2, wheat germ agglutinin (WGA) and phytohemagglutinin (PHA), bound the hormone quantitatively. The extent to which PHA bound Ep depended on the isolectin composition of the PHA. The leukoagglutinating form (L-PHA) failed to bind the hormone completely, while the erythroagglutinating form (E-PHA) had such a high affinity for Ep that it could be released only with 4 M guanidine hydrochloride (pH 7.0). PHA-P, which contains both the E and L isolectins, bound Ep quantitatively, and the hormone could be partially released by N-**acetylgalactosamine** or sialic acid. Ep bound to WGA-agarose could be partially released with N-acetylglucosamine or sialic acid; with N,N-diacetylchitobiose recovery was quantitative. Two adsorbents, Cibacron Blue F3GA and octylsuccinic anhydride, which have a high affinity for albumin, a major contaminant of crude Ep preparations, also bound Ep quantitatively. Agarose-bound antialbumin Ig[immunoglobulin]G was effective in removing albumin from crude hormone preparations without adsorbing a significant quantity of Ep. Neither agarose-bound neuraminidase nor hydrophobic interaction chromatography employing agarose

coated with substituted or unsubstituted hydrocarbon chains separated Ep
from contaminating proteins in crude preparations of the hormone.
CC Biochemical Methods - Proteins, Peptides and Amino Acids *10054
Biochemical Methods - Carbohydrates 10058
Biochemical Studies - General 10060
Biochemical Studies - Proteins, Peptides and Amino Acids 10064
Biochemical Studies - Carbohydrates 10068
Biophysics - General Biophysical Techniques 10504
Enzymes - Physiological Studies 10808
Movement 12100
Blood, Blood-Forming Organs and Body Fluids - Lymphatic Tissue and
Reticuloendothelial System *15008
Endocrine System - General *17002
Plant Physiology, Biochemistry and Biophysics - Chemical Constituents
51522
Pharmacognosy and Pharmaceutical Botany 54000
BC Plantae - Unspecified 11000
Gramineae 25305
Hominidae 86215
IT Miscellaneous Descriptors
HUMAN WHEAT GERM AGGLUTININ PHYTO HEM AGGLUTININ
RN **11096-26-7 (ERYTHROPOIETIN)**

=> d his

(FILE 'HOME' ENTERED AT 13:36:39 ON 08 NOV 2002)
SET COST OFF

FILE 'REGISTRY' ENTERED AT 13:36:51 ON 08 NOV 2002
E ERYTHROPOIETIN/CN
L1 1 S E3
E ERYTHROPOIETIN
L2 1166 S E3 NOT L1
L3 0 S L2 AND OC5/ES
L4 0 S L2 AND PMS/CI
SEL RN L1
L5 6 S E1/CRN
L6 1166 S L2,L5

FILE 'HCAPLUS' ENTERED AT 13:38:18 ON 08 NOV 2002
L7 7105 S L1
L8 359 S L6
L9 9347 S ERYTHROPOIETIN
L10 4784 S EPOETIN OR EPO
L11 11366 S L7-L10
E BURG J/AU
L12 67 S E3-E11,E24
E BUERG J/AU
E BEURG J/AU
E SELLINGER K/AU
L13 10 S E4,E5
E HASELBECK A/AU
L14 27 S E3,E4
E HAESELBECK A/AU
E HEASELBECK A/AU
E KOLL H/AU
L15 27 S E3,E4,E6-E8
E KOELL H/AU
L16 1 S E4
E KEOLL H/AU
L17 14 S L11 AND L12-L16
E DE97-19753681/AP,PRN

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L18           1 S E3,E4
                E DE98-19813415/AP,PRN
                E WO98-EP7876/AP,PRN
L19           1 S E3,E4
                E D HIS
                E EP98-113415/AP,PRN
L20           1 S E4
L21           1 S L12-L17 AND L18-L20
L22          14 S L17,L21
L23           3 S L12 AND L13-L17
L24           1 S L13 AND L14-L17
L25           0 S L15 AND L16
L26           3 S L23,L24
L27          14 S L22-L26
L28         170 S L11 (L) GLYCOSYLAT?
                E GLYCOSYLATION/CT
                E E4+ALL
L29        1817 S E2
                E GLYCOSYLATION/CT
                E E3+ALL
L30       18186 S E4,E3+NT
                E E41+ALL
L31         605 S E4,E3+NT
L32          99 S L11 AND L29-L31
L33         204 S L28,L32
L34          11 S L33 AND (ACETYLLACTOSAMINE OR ACETYL(L)LACTOSAMINE)
L35          19 S L33 AND (?ACETYLLACTOSAMIN? OR ?ACETYL?(L)?LACTOSAMIN?)
L36          19 S L34,L35

     FILE 'REGISTRY' ENTERED AT 13:51:10 ON 08 NOV 2002
L37           1 S 32181-59-2
L38         238 S C14H25NO11/MF
L39         233 S L38 AND OC5/ES
L40         192 S L39 AND ACETYLAMINO
L41          43 S L40 AND GLUCOSE
L42          17 S L41 AND GALACTO?
L43           5 S L42 AND 4 NOT (T/ELS OR 13C# OR 6)
L44           4 S L43 NOT IDS/CI
L45           3 S L44 NOT 92762-44-2
L46          12 S L42 NOT L43
                SEL RN 2 3
L47           2 S E1-E2
L48           5 S L45,L47
                SEL RN
L49           5 S E3-E7/CRN

     FILE 'HCAPLUS' ENTERED AT 13:57:48 ON 08 NOV 2002
L50         760 S L48 OR L49
L51           8 S L50 AND L11
L52           3 S L36 AND L51
L53           5 S L51 NOT L52
                SEL DN AN 3 4 5
L54           3 S E8-E16 AND L53
L55           6 S L52,L54
L56          16 S L36 NOT L55
L57          22 S L55,L56
L58          36 S L27,L57
L59          21 S L58 AND ?LACTOSAMIN?
L60          36 S L58,L59

     FILE 'REGISTRY' ENTERED AT 14:11:20 ON 08 NOV 2002
L61             STR
L62          50 S L61
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L63             STR L61
L64          9510 S L63 FUL
                 SAV L64 AUDET555/A
L65           121 S L64 AND PMS/CI
L66          9387 S L64 NOT L49,L65
L67             STR
L68            50 S L67 SAM SUB=L66
L69          1402 S L67 FUL SUB=L66
                 SAV L69 AUDET555A/A
L70             STR
L71            17 S L70 SAM SUB=L69
L72           373 S L70 FUL SUB=L69
                 SAV L72 AUDET555B/A
L73          1029 S L69 NOT L72
L74            41 S L73 AND IDS/CI
L75           988 S L73 NOT L74
L76            40 S L74 NOT SPIRO

     FILE 'HCAPLUS' ENTERED AT 14:31:54 ON 08 NOV 2002
L77           768 S L75 OR L76
L78             3 S L77 AND L11
L79            39 S L60,L78

     FILE 'REGISTRY' ENTERED AT 14:32:52 ON 08 NOV 2002
L80             STR L67
L81             STR L80
L82           511 S L81 FUL SUB=L73
                 SAV L82 AUDET555C/A
L83             STR
L84             0 S L83 SAM SUB=L82
L85             1 S L83 FUL SUB=L82
                 SAV L85 AUDET555D/A
L86             STR L67
L87            48 S L86 FUL SUB=L82
                 SAV L87 AUDET555E/A
L88             STR L86
L89            18 S L88 FUL SUB=L87
                 SAV L89 AUDET555F/A
L90             0 S L89 AND NR>=21

     FILE 'HCAPLUS' ENTERED AT 15:15:07 ON 08 NOV 2002
L91             7 S L89 OR L85

     FILE 'REGISTRY' ENTERED AT 15:15:32 ON 08 NOV 2002
L92             3 S L48 NOT L47

     FILE 'HCAPLUS' ENTERED AT 15:18:04 ON 08 NOV 2002
                 SEL HIT RN L60

     FILE 'REGISTRY' ENTERED AT 15:18:57 ON 08 NOV 2002
L93             4 S E17-E20

     FILE 'BIOSIS' ENTERED AT 15:19:17 ON 08 NOV 2002
L94         17124 S L11
L95            29 S L94 AND ?LACTOSAMIN?
L96             3 S L94 AND L48,L49
L97             0 S L94 AND L75,L76
L98             0 S L94 AND L82
L99            29 S L95,L96
L100           22 S L99 AND PY<=1997
L101            0 S L99 AND (BURG J? OR BUERG J? OR BEURG J? OR SELLINGER K? OR

     FILE 'HCAPLUS, BIOSIS' ENTERED AT 15:22:43 ON 08 NOV 2002
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L102 56 DUP REM L91 L60 L99 (16 DUPLICATES REMOVED)